



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : C12N 15/12, C07K 14/705, C12N 15/62, C07K 16/28		A2	(11) International Publication Number: <b>WO 99/63088</b>
			(43) International Publication Date: 9 December 1999 (09.12.99)
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60/087,609	2 June 1998 (02.06.98)	US	
60/087,759	2 June 1998 (02.06.98)	US	
60/087,827	3 June 1998 (03.06.98)	US	
60/088,021	4 June 1998 (04.06.98)	US	
60/088,025	4 June 1998 (04.06.98)	US	
60/088,028	4 June 1998 (04.06.98)	US	
60/088,029	4 June 1998 (04.06.98)	US	
60/088,030	4 June 1998 (04.06.98)	US	
60/088,033	4 June 1998 (04.06.98)	US	
60/088,326	4 June 1998 (04.06.98)	US	
60/088,167	5 June 1998 (05.06.98)	US	
60/088,202	5 June 1998 (05.06.98)	US	
60/088,212	5 June 1998 (05.06.98)	US	
60/088,217	5 June 1998 (05.06.98)	US	
60/088,655	9 June 1998 (09.06.98)	US	
60/088,722	10 June 1998 (10.06.98)	US	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
60/088,730	10 June 1998 (10.06.98)	US	
60/088,734	10 June 1998 (10.06.98)	US	
60/088,738	10 June 1998 (10.06.98)	US	
60/088,740	10 June 1998 (10.06.98)	US	Published Without international search report and to be republished upon receipt of that report.
60/088,741	10 June 1998 (10.06.98)	US	

(Continued after the drawings)

(54) Title: MEMBRANE-BOUND PROTEINS AND NUCLEIC ACIDS ENCODING THE SAME

## (57) Abstract

The present invention is directed to polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

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EE	Estonia						

## NOVEL POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME

### FIELD OF THE INVENTION

The present invention relates generally to the identification and isolation of novel DNA and to the recombinant production of novel polypeptides.

5

### BACKGROUND OF THE INVENTION

Extracellular proteins play important roles in, among other things, the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment.

Secreted proteins have various industrial applications, including as pharmaceuticals, diagnostics, biosensors and bioreactors. Most protein drugs available at present, such as thrombolytic agents, interferons, interleukins, erythropoietins, colony stimulating factors, and various other cytokines, are secretory proteins. Their receptors, which are membrane proteins, also have potential as therapeutic or diagnostic agents. Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. Examples of screening methods and techniques are described in the literature [see, for example, Klein et al., *Proc. Natl. Acad. Sci.* 93:7108-7113 (1996); U.S. Patent No. 5,536,637].

Membrane-bound proteins and receptors can play important roles in, among other things, the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. Such membrane-bound proteins and cell receptors include, but are not limited to, cytokine receptors, receptor kinases, receptor phosphatases, receptors involved in cell-cell interactions, and cellular adhesion molecules like selectins and integrins. For instance, transduction of signals that regulate cell growth and differentiation is regulated in part by phosphorylation of various cellular proteins. Protein tyrosine kinases, enzymes that catalyze that process, can also act as growth factor receptors. Examples include fibroblast growth factor receptor and nerve growth factor receptor.

Membrane-bound proteins and receptor molecules have various industrial applications, including as pharmaceutical and diagnostic agents. Receptor immunoadhesins, for instance, can be employed as therapeutic agents to block receptor-ligand interactions. The membrane-bound proteins can also be employed for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction.

Efforts are being undertaken by both industry and academia to identify new, native receptor or membrane-bound proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel receptor or membrane-bound proteins.

1. **PRO281**

A novel gene designated testis enhanced gene transcript (TEGT) has recently been identified in humans (Walter et al., Genomics 20:301-304 (1995)). Recent results have shown that TEGT protein is developmentally regulated in the mammalian testis and possesses a nuclear targeting motif that allows the protein to localize to the nucleus (Walter et al., Mamm. Genome 5:216-221 (1994)). As such, it is believed that the TEGT protein plays an important role in testis development. There is, therefore, substantial interest in identifying and characterizing novel polypeptides having homology to the TEGT protein. We herein describe the identification and characterization of novel polypeptides having homology to TEGT protein, designated herein as PRO281 polypeptides.

2. **PRO276**

Efforts are being undertaken by both industry and academia to identify new, native membrane-bound proteins. Many of these efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel membrane-bound proteins. We herein describe the identification and characterization of novel transmembrane polypeptides, designated herein as PRO276 polypeptides.

3. **PRO189**

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many of these efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. We herein describe the identification and characterization of novel secreted polypeptides, designated herein as PRO189 polypeptides.

4. **PRO190**

Of particular interest are proteins having seven transmembrane domains (7TM), or more generally, all multiple transmembrane spanning proteins. Among multiple transmembrane spanning proteins are ion channels and transporters. Examples of transporters are the UDP-galactose transporter described in Ishida, et al., J. Biochem., 120(6):1074-1078 (1996), and the CMP-sialic acid transporter described in Eckhardt, et al., PNAS, 93(15):7572-7576 (1996). We herein describe the identification and characterization of novel transmembrane polypeptides, designated herein as PRO190 polypeptides.

sequence of amino acid residues 1 to about 229, inclusive of Figure 306 (SEQ ID NO:424).

In a further aspect, the invention concerns an isolated PRO1384 polypeptide, comprising an amino acid sequence scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 1 to 229 of Figure 306 (SEQ ID NO:424).

5 In yet another aspect, the invention concerns an isolated PRO1384 polypeptide, comprising the sequence of amino acid residues 1 to about 229, inclusive of Figure 306 (SEQ ID NO:424), or a fragment thereof sufficient to provide a binding site for an anti-PRO1384 antibody. Preferably, the PRO1384 fragment retains a qualitative biological activity of a native PRO1384 polypeptide.

10 In a still further aspect, the invention provides a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO1384 polypeptide having the sequence of amino acid residues from about 1 to about 229, inclusive of Figure 306 (SEQ ID NO:424), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has at least about an 80% sequence identity, preferably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), (ii) culturing a host cell comprising  
15 the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO1384 polypeptide. In a particular embodiment, the agonist or antagonist is an anti-PRO1384 antibody.

20 In a further embodiment, the invention concerns a method of identifying agonists or antagonists of a native PRO1384 polypeptide, by contacting the native PRO1384 polypeptide with a candidate molecule and monitoring a biological activity mediated by said polypeptide.

In a still further embodiment, the invention concerns a composition comprising a PRO1384 polypeptide, or an agonist or antagonist as hereinabove defined, in combination with a pharmaceutically acceptable carrier.

### 25 136. Additional Embodiments

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the above or below described polypeptides. A host cell comprising any such vector is also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the above or below described polypeptides is further provided and comprises culturing host cells under  
30 conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the above or below described polypeptides fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises any of the above or below described polypeptides fused to an epitope tag sequence  
35 or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences, wherein those probes may be derived from any of the above or below described nucleotide sequences.

In other embodiments, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a PRO polypeptide.

5 In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence  
10 identity, yet more preferably at least about 98% sequence identity and yet more preferably at least about 99% sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, a full-length amino acid sequence lacking the signal peptide as disclosed herein or an extracellular domain of a transmembrane protein as disclosed herein, or (b) the complement of the DNA molecule of (a).

20 In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence  
25 identity, yet more preferably at least about 98% sequence identity and yet more preferably at least about 99% sequence identity to (a) a DNA molecule having the coding sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a full-length PRO polypeptide lacking the signal peptide as disclosed herein or the coding sequence of an extracellular domain of a transmembrane PRO polypeptide as disclosed herein, or (b) the complement of the DNA molecule of (a).

35 In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence

identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity and yet more preferably at least about 99% sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein, or (b) the complement of the DNA molecule of (a).

Another aspect the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptide are disclosed herein. Therefore, soluble extracellular domains of the herein described PRO polypeptides are contemplated.

Another embodiment is directed to fragments of a PRO polypeptide coding sequence that may find use as, for example, hybridization probes or for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO antibody. Such nucleic acid fragments are usually at least about 20 nucleotides in length, preferably at least about 30 nucleotides in length, more preferably at least about 40 nucleotides in length, yet more preferably at least about 50 nucleotides in length, yet more preferably at least about 60 nucleotides in length, yet more preferably at least about 70 nucleotides in length, yet more preferably at least about 80 nucleotides in length, yet more preferably at least about 90 nucleotides in length, yet more preferably at least about 100 nucleotides in length, yet more preferably at least about 110 nucleotides in length, yet more preferably at least about 120 nucleotides in length, yet more preferably at least about 130 nucleotides in length, yet more preferably at least about 140 nucleotides in length, yet more preferably at least about 150 nucleotides in length, yet more preferably at least about 160 nucleotides in length, yet more preferably at least about 170 nucleotides in length, yet more preferably at least about 180 nucleotides in length, yet more preferably at least about 190 nucleotides in length, yet more preferably at least about 200 nucleotides in length, yet more preferably at least about 250 nucleotides in length, yet more preferably at least about 300 nucleotides in length, yet more preferably at least about 350 nucleotides in length, yet more preferably at least about 400 nucleotides in length, yet more preferably at least about 450 nucleotides in length, yet more preferably at least about 500 nucleotides in length, yet more preferably at least about 600 nucleotides in length, yet more preferably at least about 700 nucleotides in length, yet more preferably at least about 800 nucleotides in length, yet more preferably at least about 900 nucleotides in length and yet more preferably at least about 1000 nucleotides in length, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length. It is noted that novel fragments of a PRO polypeptide-encoding nucleotide sequence may be determined in a routine manner by aligning the PRO polypeptide-encoding nucleotide

sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding nucleotide sequences are contemplated herein. Also contemplated are the PRO polypeptide fragments encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

- 5 In another embodiment, the invention provides isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences hereinabove identified.

- In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet  
10 more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least  
15 about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity and yet more preferably at least about 99% sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, a full-length amino acid sequence lacking the signal peptide as disclosed herein or an  
20 extracellular domain of a transmembrane protein as disclosed herein.

- In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity,  
25 yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at  
30 least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity and yet more preferably at least about 99% sequence identity to an amino acid sequence encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein.

- In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid  
35 sequence scoring at least about 80% positives, preferably at least about 81% positives, more preferably at least about 82% positives, yet more preferably at least about 83% positives, yet more preferably at least about 84% positives, yet more preferably at least about 85% positives, yet more preferably at least about 86% positives,



yet more preferably at least about 87% positives, yet more preferably at least about 88% positives, yet more preferably at least about 89% positives, yet more preferably at least about 90% positives, yet more preferably at least about 91% positives, yet more preferably at least about 92% positives, yet more preferably at least about 93% positives, yet more preferably at least about 94% positives, yet more preferably at least about 95% positives, yet more preferably at least about 96% positives, yet more preferably at least about 97% positives, yet more preferably at least about 98% positives and yet more preferably at least about 99% positives when compared with the amino acid sequence of a PRO polypeptide having a full-length amino acid sequence as disclosed herein, a full-length amino acid sequence lacking the signal peptide as disclosed herein or an extracellular domain of a transmembrane protein as disclosed herein.

In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as hereinbefore described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

Another aspect the invention provides an isolated PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as hereinbefore described, or an anti-PRO antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a nucleotide sequence (SEQ ID NO:1) of a native sequence PRO281 (UNQ244) cDNA, wherein SEQ ID NO:1 is a clone designated herein as "DNA16422-1209".

Figure 2 shows the amino acid sequence (SEQ ID NO:2) derived from the coding sequence of SEQ ID NO:1 shown in Figure 1.

Figure 3 shows a nucleotide sequence (SEQ ID NO:5) of a native sequence PRO276 (UNQ243) cDNA, wherein SEQ ID NO:5 is a clone designated herein as "DNA16435-1208".

Figure 4 shows the amino acid sequence (SEQ ID NO:6) derived from the coding sequence of SEQ ID NO:5 shown in Figure 3.

Figure 5 shows a nucleotide sequence (SEQ ID NO:7) of a native sequence PRO189 (UNQ163) cDNA, wherein SEQ ID NO:7 is a clone designated herein as "DNA21642-1391".

Figure 6 shows the amino acid sequence (SEQ ID NO:8) derived from the coding sequence of SEQ ID NO:7 shown in Figure 5.

Figure 7 shows a nucleotide sequence designated herein as DNA14187 (SEQ ID NO:9).

Figure 8 shows a nucleotide sequence (SEQ ID NO:13) of a native sequence PRO190 (UNQ164) cDNA, wherein SEQ ID NO:13 is a clone designated herein as "DNA23334-1392".

Figure 9 shows the amino acid sequence (SEQ ID NO:14) derived from the coding sequence of SEQ ID NO:13 shown in Figure 8.

5        Figure 10 shows a nucleotide sequence designated herein as DNA14232 (SEQ ID NO:15).

Figure 11 shows a nucleotide sequence (SEQ ID NO:19) of a native sequence PRO341 (UNQ300) cDNA, wherein SEQ ID NO:19 is a clone designated herein as "DNA26288-1239".

Figure 12 shows the amino acid sequence (SEQ ID NO:20) derived from the coding sequence of SEQ ID NO:19 shown in Figure 11.

10       Figure 13 shows a nucleotide sequence designated herein as DNA12920 (SEQ ID NO:21).

Figure 14 shows a nucleotide sequence (SEQ ID NO:22) of a native sequence PRO180 (UNQ154) cDNA, wherein SEQ ID NO:22 is a clone designated herein as "DNA26843-1389".

Figure 15 shows the amino acid sequence (SEQ ID NO:23) derived from the coding sequence of SEQ ID NO:22 shown in Figure 14.

15       Figure 16 shows a nucleotide sequence designated herein as DNA12922 (SEQ ID NO:24).

Figure 17 shows a nucleotide sequence (SEQ ID NO:27) of a native sequence PRO194 (UNQ168) cDNA, wherein SEQ ID NO:27 is a clone designated herein as "DNA26844-1394".

Figure 18 shows the amino acid sequence (SEQ ID NO:28) derived from the coding sequence of SEQ ID NO:27 shown in Figure 17.

20       Figure 19 shows a nucleotide sequence (SEQ ID NO:29) of a native sequence PRO203 (UNQ177) cDNA, wherein SEQ ID NO:29 is a clone designated herein as "DNA30862-1396".

Figure 20 shows the amino acid sequence (SEQ ID NO:30) derived from the coding sequence of SEQ ID NO:29 shown in Figure 19.

Figure 21 shows a nucleotide sequence designated herein as DNA15618 (SEQ ID NO:31).

25       Figure 22 shows a nucleotide sequence (SEQ ID NO:32) of a native sequence PRO290 (UNQ253) cDNA, wherein SEQ ID NO:32 is a clone designated herein as "DNA35680-1212".

Figure 23 shows the amino acid sequence (SEQ ID NO:33) derived from the coding sequence of SEQ ID NO:32 shown in Figure 22.

30       Figure 24 shows a nucleotide sequence (SEQ ID NO:35) of a native sequence PRO874 (UNQ441) cDNA, wherein SEQ ID NO:35 is a clone designated herein as "DNA40621-1440".

Figure 25 shows the amino acid sequence (SEQ ID NO:36) derived from the coding sequence of SEQ ID NO:35 shown in Figure 24.

Figure 26 shows a nucleotide sequence (SEQ ID NO:40) of a native sequence PRO710 (UNQ374) cDNA, wherein SEQ ID NO:40 is a clone designated herein as "DNA44161-1434".

35       Figure 27 shows the amino acid sequence (SEQ ID NO:41) derived from the coding sequence of SEQ ID NO:40 shown in Figure 26.

Figure 28 shows a nucleotide sequence designated herein as DNA38190 (SEQ ID NO:42).

Figure 29 shows a nucleotide sequence (SEQ ID NO:46) of a native sequence PRO1151 (UNQ581) cDNA, wherein SEQ ID NO:46 is a clone designated herein as "DNA44694-1500".

Figure 30 shows the amino acid sequence (SEQ ID NO:47) derived from the coding sequence of SEQ ID NO:46 shown in Figure 29.

Figure 31 shows a nucleotide sequence (SEQ ID NO:51) of a native sequence PRO1282 (UNQ652) cDNA, wherein SEQ ID NO:51 is a clone designated herein as "DNA45495-1550".

Figure 32 shows the amino acid sequence (SEQ ID NO:52) derived from the coding sequence of SEQ ID NO:51 shown in Figure 31.

Figure 33 shows a nucleotide sequence (SEQ ID NO:56) of a native sequence PRO358 cDNA, wherein SEQ ID NO:56 is a clone designated herein as "DNA47361-1154".

Figure 34 shows the amino acid sequence (SEQ ID NO:57) derived from the coding sequence of SEQ ID NO:56 shown in Figure 33.

Figures 35A-B show a nucleotide sequence (SEQ ID NO:61) of a native sequence PRO1310 cDNA, wherein SEQ ID NO:61 is a clone designated herein as "DNA47394-1572".

Figure 36 shows the amino acid sequence (SEQ ID NO:62) derived from the coding sequence of SEQ ID NO:61 shown in Figures 35A-B.

Figure 37 shows a nucleotide sequence (SEQ ID NO:66) of a native sequence PRO698 (UNQ362) cDNA, wherein SEQ ID NO:66 is a clone designated herein as "DNA48320-1433".

Figure 38 shows the amino acid sequence (SEQ ID NO:67) derived from the coding sequence of SEQ ID NO:66 shown in Figure 37.

Figure 39 shows a nucleotide sequence designated herein as DNA39906 (SEQ ID NO:68).

Figure 40 shows a nucleotide sequence (SEQ ID NO:72) of a native sequence PRO732 (UNQ396) cDNA, wherein SEQ ID NO:72 is a clone designated herein as "DNA48334-1435".

Figure 41 shows the amino acid sequence (SEQ ID NO:73) derived from the coding sequence of SEQ ID NO:72 shown in Figure 40.

Figure 42 shows a nucleotide sequence designated herein as DNA20239 (SEQ ID NO:74).

Figure 43 shows a nucleotide sequence designated herein as DNA38050 (SEQ ID NO:75).

Figure 44 shows a nucleotide sequence designated herein as DNA40683 (SEQ ID NO:76).

Figure 45 shows a nucleotide sequence designated herein as DNA42580 (SEQ ID NO:77).

Figures 46A-B show a nucleotide sequence (SEQ ID NO:83) of a native sequence PRO1120 (UNQ559) cDNA, wherein SEQ ID NO:83 is a clone designated herein as "DNA48606-1479".

Figure 47 shows the amino acid sequence (SEQ ID NO:84) derived from the coding sequence of SEQ ID NO:83 shown in Figures 46A-B.

Figure 48 shows a nucleotide sequence (SEQ ID NO:94) of a native sequence PRO537 (UNQ338) cDNA, wherein SEQ ID NO:94 is a clone designated herein as "DNA49141-1431".

Figure 49 shows the amino acid sequence (SEQ ID NO:95) derived from the coding sequence of SEQ ID NO:94 shown in Figure 48.

Figure 50 shows a nucleotide sequence (SEQ ID NO:96) of a native sequence PRO536 (UNQ337)

cDNA, wherein SEQ ID NO:96 is a clone designated herein as "DNA49142-1430".

Figure 51 shows the amino acid sequence (SEQ ID NO:97) derived from the coding sequence of SEQ ID NO:96 shown in Figure 50.

Figure 52 shows a nucleotide sequence (SEQ ID NO:98) of a native sequence PRO535 (UNQ336) cDNA, wherein SEQ ID NO:98 is a clone designated herein as "DNA49143-1429".

5        Figure 53 shows the amino acid sequence (SEQ ID NO:99) derived from the coding sequence of SEQ ID NO:98 shown in Figure 52.

Figure 54 shows a nucleotide sequence designated herein as DNA30861 (SEQ ID NO:100).

Figure 55 shows a nucleotide sequence designated herein as DNA36351 (SEQ ID NO:101).

10        Figure 56 shows a nucleotide sequence (SEQ ID NO:102) of a native sequence PRO718 (UNQ386) cDNA, wherein SEQ ID NO:102 is a clone designated herein as "DNA49647-1398".

Figure 57 shows the amino acid sequence (SEQ ID NO:103) derived from the coding sequence of SEQ ID NO:102 shown in Figure 56.

Figure 58 shows a nucleotide sequence designated herein as DNA15386 (SEQ ID NO:104).

Figure 59 shows a nucleotide sequence designated herein as DNA16630 (SEQ ID NO:105).

15        Figure 60 shows a nucleotide sequence designated herein as DNA16829 (SEQ ID NO:106).

Figure 61 shows a nucleotide sequence designated herein as DNA28357 (SEQ ID NO:107).

Figure 62 shows a nucleotide sequence designated herein as DNA43512 (SEQ ID NO:108).

Figure 63 shows a nucleotide sequence (SEQ ID NO:112) of a native sequence PRO872 (UNQ439) cDNA, wherein SEQ ID NO:112 is a clone designated herein as "DNA49819-1439".

20        Figure 64 shows the amino acid sequence (SEQ ID NO:113) derived from the coding sequence of SEQ ID NO:112 shown in Figure 63.

Figure 65 shows a nucleotide sequence (SEQ ID NO:114) of a native sequence PRO1063 (UNQ128) cDNA, wherein SEQ ID NO:114 is a clone designated herein as "DNA49820-1427".

25        Figure 66 shows the amino acid sequence (SEQ ID NO:115) derived from the coding sequence of SEQ ID NO:114 shown in Figure 65.

Figure 67 shows a nucleotide sequence (SEQ ID NO:116) of a native sequence PRO619 (UNQ355) cDNA, wherein SEQ ID NO:116 is a clone designated herein as "DNA49821-1562".

Figure 68 shows the amino acid sequence (SEQ ID NO:117) derived from the coding sequence of SEQ ID NO:116 shown in Figure 67.

30        Figure 69 shows a nucleotide sequence (SEQ ID NO:118) of a native sequence PRO943 (UNQ480) cDNA, wherein SEQ ID NO:118 is a clone designated herein as "DNA52192-1369".

Figure 70 shows the amino acid sequence (SEQ ID NO:119) derived from the coding sequence of SEQ ID NO:118 shown in Figure 69.

35        Figure 71 shows a nucleotide sequence (SEQ ID NO:123) of a native sequence PRO1188 (UNQ602) cDNA, wherein SEQ ID NO:123 is a clone designated herein as "DNA52598-1518".

Figure 72 shows the amino acid sequence (SEQ ID NO:124) derived from the coding sequence of SEQ ID NO:123 shown in Figure 71.

Figure 73 shows a nucleotide sequence (SEQ ID NO:128) of a native sequence PRO1133 (UNQ571) cDNA, wherein SEQ ID NO:128 is a clone designated herein as "DNA53913-1490".

Figure 74 shows the amino acid sequence (SEQ ID NO:129) derived from the coding sequence of SEQ ID NO:128 shown in Figure 73.

Figure 75 shows a nucleotide sequence (SEQ ID NO:134) of a native sequence PRO784 (UNQ459) cDNA, wherein SEQ ID NO:134 is a clone designated herein as "DNA53978-1443".

Figure 76 shows the amino acid sequence (SEQ ID NO:135) derived from the coding sequence of SEQ ID NO:134 shown in Figure 75.

Figure 77 shows a nucleotide sequence designated herein as DNA44661 (SEQ ID NO:136).

Figure 78 shows a nucleotide sequence (SEQ ID NO:137) of a native sequence PRO783 (UNQ458) cDNA, wherein SEQ ID NO:137 is a clone designated herein as "DNA53996-1442".

Figure 79 shows the amino acid sequence (SEQ ID NO:138) derived from the coding sequence of SEQ ID NO:137 shown in Figure 78.

Figure 80 shows a nucleotide sequence designated herein as DNA45201 (SEQ ID NO:139).

Figure 81 shows a nucleotide sequence designated herein as DNA14575 (SEQ ID NO:140).

Figure 82 shows a nucleotide sequence (SEQ ID NO:145) of a native sequence PRO820 (UNQ503) cDNA, wherein SEQ ID NO:145 is a clone designated herein as "DNA56041-1416".

Figure 83 shows the amino acid sequence (SEQ ID NO:146) derived from the coding sequence of SEQ ID NO:145 shown in Figure 82.

Figure 84 shows a nucleotide sequence (SEQ ID NO:147) of a native sequence PRO1080 (UNQ537) cDNA, wherein SEQ ID NO:147 is a clone designated herein as "DNA56047-1456".

Figure 85 shows the amino acid sequence (SEQ ID NO:148) derived from the coding sequence of SEQ ID NO:147 shown in Figure 84.

Figure 86 shows a nucleotide sequence designated herein as DNA36527 (SEQ ID NO:149).

Figure 87 shows a nucleotide sequence (SEQ ID NO:150) of a native sequence PRO1079 (UNQ536) cDNA, wherein SEQ ID NO:150 is a clone designated herein as "DNA56050-1455".

Figure 88 shows the amino acid sequence (SEQ ID NO:151) derived from the coding sequence of SEQ ID NO:150 shown in Figure 87.

Figure 89 shows a nucleotide sequence (SEQ ID NO:152) of a native sequence PRO793 (UNQ432) cDNA, wherein SEQ ID NO:152 is a clone designated herein as "DNA56110-1437".

Figure 90 shows the amino acid sequence (SEQ ID NO:153) derived from the coding sequence of SEQ ID NO:152 shown in Figure 89.

Figure 91 shows a nucleotide sequence designated herein as DNA50177 (SEQ ID NO:154).

Figure 92 shows a nucleotide sequence (SEQ ID NO:155) of a native sequence PRO1016 (UNQ499) cDNA, wherein SEQ ID NO:155 is a clone designated herein as "DNA56113-1378".

Figure 93 shows the amino acid sequence (SEQ ID NO:156) derived from the coding sequence of SEQ ID NO:155 shown in Figure 92.

Figure 94 shows a nucleotide sequence (SEQ ID NO:157) of a native sequence PRO1013 (UNQ496) cDNA, wherein SEQ ID NO:157 is a clone designated herein as "DNA56410-1414".

Figure 95 shows the amino acid sequence (SEQ ID NO:158) derived from the coding sequence of SEQ ID NO:157 shown in Figure 94.

Figure 96 shows a nucleotide sequence (SEQ ID NO:159) of a native sequence PRO937 (UNQ474) cDNA, wherein SEQ ID NO:159 is a clone designated herein as "DNA56436-1448".

Figure 97 shows the amino acid sequence (SEQ ID NO:160) derived from the coding sequence of SEQ ID NO:159 shown in Figure 96.

Figure 98 shows a nucleotide sequence (SEQ ID NO:164) of a native sequence PRO842 (UNQ473) cDNA, wherein SEQ ID NO:164 is a clone designated herein as "DNA56855-1447".

Figure 99 shows the amino acid sequence (SEQ ID NO:165) derived from the coding sequence of SEQ ID NO:164 shown in Figure 98.

Figure 100 shows a nucleotide sequence (SEQ ID NO:166) of a native sequence PRO839 (UNQ472) cDNA, wherein SEQ ID NO:166 is a clone designated herein as "DNA56859-1445".

Figure 101 shows the amino acid sequence (SEQ ID NO:167) derived from the coding sequence of SEQ ID NO:166 shown in Figure 100.

Figure 102 shows a nucleotide sequence (SEQ ID NO:168) of a native sequence PRO1180 (UNQ594) cDNA, wherein SEQ ID NO:168 is a clone designated herein as "DNA56860-1510".

Figure 103 shows the amino acid sequence (SEQ ID NO:169) derived from the coding sequence of SEQ ID NO:168 shown in Figure 102.

Figure 104 shows a nucleotide sequence (SEQ ID NO:170) of a native sequence PRO1134 (UNQ572) cDNA, wherein SEQ ID NO:170 is a clone designated herein as "DNA56865-1491".

Figure 105 shows the amino acid sequence (SEQ ID NO:171) derived from the coding sequence of SEQ ID NO:170 shown in Figure 104.

Figure 106 shows a nucleotide sequence designated herein as DNA52352 (SEQ ID NO:172).

Figure 107 shows a nucleotide sequence designated herein as DNA55725 (SEQ ID NO:173).

Figure 108 shows a nucleotide sequence (SEQ ID NO:174) of a native sequence PRO830 (UNQ470) cDNA, wherein SEQ ID NO:174 is a clone designated herein as "DNA56866-1342".

Figure 109 shows the amino acid sequence (SEQ ID NO:175) derived from the coding sequence of SEQ ID NO:174 shown in Figure 108.

Figure 110 shows a nucleotide sequence (SEQ ID NO:176) of a native sequence PRO1115 (UNQ558) cDNA, wherein SEQ ID NO:176 is a clone designated herein as "DNA56868-1478".

Figure 111 shows the amino acid sequence (SEQ ID NO:177) derived from the coding sequence of SEQ ID NO:176 shown in Figure 110.

Figure 112 shows a nucleotide sequence (SEQ ID NO:178) of a native sequence PRO1277 (UNQ647) cDNA, wherein SEQ ID NO:178 is a clone designated herein as "DNA56869-1545".

Figure 113 shows the amino acid sequence (SEQ ID NO:179) derived from the coding sequence of SEQ ID NO:178 shown in Figure 112.

Figure 114 shows a nucleotide sequence (SEQ ID NO:180) of a native sequence PRO1135 (UNQ573) cDNA, wherein SEQ ID NO:180 is a clone designated herein as "DNA56870-1492".

Figure 115 shows the amino acid sequence (SEQ ID NO:181) derived from the coding sequence of SEQ ID NO:180 shown in Figure 114.

Figure 116 shows a nucleotide sequence (SEQ ID NO:182) of a native sequence PRO1114 (UNQ557) cDNA, wherein SEQ ID NO:182 is a clone designated herein as "DNA57033-1403".

Figure 117 shows the amino acid sequence (SEQ ID NO:183) derived from the coding sequence of SEQ ID NO:182 shown in Figure 116.

Figure 118 shows a nucleotide sequence designated herein as DNA48466 (SEQ ID NO:184).

Figure 119 shows a nucleotide sequence (SEQ ID NO:188) of a native sequence PRO828 (UNQ469) cDNA, wherein SEQ ID NO:188 is a clone designated herein as "DNA57037-1444".

Figure 120 shows the amino acid sequence (SEQ ID NO:189) derived from the coding sequence of SEQ ID NO:188 shown in Figure 119.

Figure 121 shows a nucleotide sequence (SEQ ID NO:193) of a native sequence PRO1009 (UNQ493) cDNA, wherein SEQ ID NO:193 is a clone designated herein as "DNA57129-1413".

Figure 122 shows the amino acid sequence (SEQ ID NO:194) derived from the coding sequence of SEQ ID NO:193 shown in Figure 121.

Figure 123 shows a nucleotide sequence designated herein as DNA50853 (SEQ ID NO:195).

Figure 124 shows a nucleotide sequence (SEQ ID NO:196) of a native sequence PRO1007 (UNQ491) cDNA, wherein SEQ ID NO:196 is a clone designated herein as "DNA57690-1374".

Figure 125 shows the amino acid sequence (SEQ ID NO:197) derived from the coding sequence of SEQ ID NO:196 shown in Figure 124.

Figure 126 shows a nucleotide sequence (SEQ ID NO:198) of a native sequence PRO1056 (UNQ521) cDNA, wherein SEQ ID NO:198 is a clone designated herein as "DNA57693-1424".

Figure 127 shows the amino acid sequence (SEQ ID NO:199) derived from the coding sequence of SEQ ID NO:198 shown in Figure 126.

Figure 128 shows a nucleotide sequence (SEQ ID NO:200) of a native sequence PRO826 (UNQ467) cDNA, wherein SEQ ID NO:200 is a clone designated herein as "DNA57694-1341".

Figure 129 shows the amino acid sequence (SEQ ID NO:201) derived from the coding sequence of SEQ ID NO:200 shown in Figure 128.

Figure 130 shows a nucleotide sequence (SEQ ID NO:202) of a native sequence PRO819 (UNQ466) cDNA, wherein SEQ ID NO:202 is a clone designated herein as "DNA57695-1340".

Figure 131 shows the amino acid sequence (SEQ ID NO:203) derived from the coding sequence of SEQ ID NO:202 shown in Figure 130.

Figure 132 shows a nucleotide sequence (SEQ ID NO:204) of a native sequence PRO1006 (UNQ490) cDNA, wherein SEQ ID NO:204 is a clone designated herein as "DNA57699-1412".

Figure 133 shows the amino acid sequence (SEQ ID NO:205) derived from the coding sequence of SEQ ID NO:204 shown in Figure 132.

Figure 134 shows a nucleotide sequence (SEQ ID NO:206) of a native sequence PRO1112 (UNQ555) cDNA, wherein SEQ ID NO:206 is a clone designated herein as "DNA57702-1476".

Figure 135 shows the amino acid sequence (SEQ ID NO:207) derived from the coding sequence of SEQ ID NO:206 shown in Figure 134.

Figure 136 shows a nucleotide sequence (SEQ ID NO:208) of a native sequence PRO1074 (UNQ531) cDNA, wherein SEQ ID NO:208 is a clone designated herein as "DNA57704-1452".

Figure 137 shows the amino acid sequence (SEQ ID NO:209) derived from the coding sequence of SEQ ID NO:208 shown in Figure 136.

Figure 138 shows a nucleotide sequence (SEQ ID NO:210) of a native sequence PRO1005 (UNQ489) cDNA, wherein SEQ ID NO:210 is a clone designated herein as "DNA57708-1005".

Figure 139 shows the amino acid sequence (SEQ ID NO:211) derived from the coding sequence of SEQ ID NO:210 shown in Figure 138.

Figure 140 shows a nucleotide sequence (SEQ ID NO:212) of a native sequence PRO1073 (UNQ530) cDNA, wherein SEQ ID NO:212 is a clone designated herein as "DNA57710-1451".

Figure 141 shows the amino acid sequence (SEQ ID NO:213) derived from the coding sequence of SEQ ID NO:212 shown in Figure 140.

Figure 142 shows a nucleotide sequence designated herein as DNA55938 (SEQ ID NO:214).

Figure 143 shows a nucleotide sequence (SEQ ID NO:215) of a native sequence PRO1152 (UNQ582) cDNA, wherein SEQ ID NO:215 is a clone designated herein as "DNA57711-1501".

Figure 144 shows the amino acid sequence (SEQ ID NO:216) derived from the coding sequence of SEQ ID NO:215 shown in Figure 143.

Figure 145 shows a nucleotide sequence designated herein as DNA55807 (SEQ ID NO:217).

Figure 146 shows a nucleotide sequence (SEQ ID NO:218) of a native sequence PRO1136 (UNQ574) cDNA, wherein SEQ ID NO:218 is a clone designated herein as "DNA57827-1493".

Figure 147 shows the amino acid sequence (SEQ ID NO:219) derived from the coding sequence of SEQ ID NO:218 shown in Figure 146.

Figure 148 shows a nucleotide sequence (SEQ ID NO:220) of a native sequence PRO813 (UNQ465) cDNA, wherein SEQ ID NO:220 is a clone designated herein as "DNA57834-1339".

Figure 149 shows the amino acid sequence (SEQ ID NO:221) derived from the coding sequence of SEQ ID NO:220 shown in Figure 148.

Figure 150 shows a nucleotide sequence (SEQ ID NO:222) of a native sequence PRO809 (UNQ464) cDNA, wherein SEQ ID NO:222 is a clone designated herein as "DNA57836-1338".

Figure 151 shows the amino acid sequence (SEQ ID NO:223) derived from the coding sequence of SEQ ID NO:222 shown in Figure 150.

Figure 152 shows a nucleotide sequence (SEQ ID NO:224) of a native sequence PRO791 (UNQ463) cDNA, wherein SEQ ID NO:224 is a clone designated herein as "DNA57838-1337".

Figure 153 shows the amino acid sequence (SEQ ID NO:225) derived from the coding sequence of SEQ ID NO:224 shown in Figure 152.



Figure 154 shows a nucleotide sequence (SEQ ID NO:226) of a native sequence PRO1004 (UNQ488) cDNA, wherein SEQ ID NO:226 is a clone designated herein as "DNA57844-1410".

Figure 155 shows the amino acid sequence (SEQ ID NO:227) derived from the coding sequence of SEQ ID NO:226 shown in Figure 154.

Figure 156 shows a nucleotide sequence (SEQ ID NO:228) of a native sequence PRO1111 (UNQ554) cDNA, wherein SEQ ID NO:228 is a clone designated herein as "DNA58721-1475".

Figure 157 shows the amino acid sequence (SEQ ID NO:229) derived from the coding sequence of SEQ ID NO:228 shown in Figure 156.

Figure 158 shows a nucleotide sequence (SEQ ID NO:230) of a native sequence PRO1344 (UNQ699) cDNA, wherein SEQ ID NO:230 is a clone designated herein as "DNA58723-1588".

Figure 159 shows the amino acid sequence (SEQ ID NO:231) derived from the coding sequence of SEQ ID NO:230 shown in Figure 158.

Figure 160 shows a nucleotide sequence (SEQ ID NO:235) of a native sequence PRO1109 (UNQ552) cDNA, wherein SEQ ID NO:235 is a clone designated herein as "DNA58737-1473".

Figure 161 shows the amino acid sequence (SEQ ID NO:236) derived from the coding sequence of SEQ ID NO:235 shown in Figure 160.

Figure 162 shows a nucleotide sequence (SEQ ID NO:240) of a native sequence PRO1383 (UNQ719) cDNA, wherein SEQ ID NO:240 is a clone designated herein as "DNA58743-1609".

Figure 163 shows the amino acid sequence (SEQ ID NO:241) derived from the coding sequence of SEQ ID NO:240 shown in Figure 162.

Figure 164 shows a nucleotide sequence (SEQ ID NO:245) of a native sequence PRO1003 (UNQ487) cDNA, wherein SEQ ID NO:245 is a clone designated herein as "DNA58846-1409".

Figure 165 shows the amino acid sequence (SEQ ID NO:246) derived from the coding sequence of SEQ ID NO:245 shown in Figure 164.

Figure 166 shows a nucleotide sequence (SEQ ID NO:247) of a native sequence PRO1108 (UNQ551) cDNA, wherein SEQ ID NO:247 is a clone designated herein as "DNA58848-1472".

Figure 167 shows the amino acid sequence (SEQ ID NO:248) derived from the coding sequence of SEQ ID NO:247 shown in Figure 166.

Figure 168 shows a nucleotide sequence (SEQ ID NO:249) of a native sequence PRO1137 (UNQ575) cDNA, wherein SEQ ID NO:249 is a clone designated herein as "DNA58849-1494".

Figure 169 shows the amino acid sequence (SEQ ID NO:250) derived from the coding sequence of SEQ ID NO:249 shown in Figure 168.

Figure 170 shows a nucleotide sequence (SEQ ID NO:252) of a native sequence PRO1138 (UNQ576) cDNA, wherein SEQ ID NO:252 is a clone designated herein as "DNA58850-1495".

Figure 171 shows the amino acid sequence (SEQ ID NO:253) derived from the coding sequence of SEQ ID NO:252 shown in Figure 170.

Figure 172 shows a nucleotide sequence designated herein as DNA49140 (SEQ ID NO:254).

Figure 173 shows a nucleotide sequence (SEQ ID NO:255) of a native sequence PRO1054 (UNQ519) cDNA, wherein SEQ ID NO:255 is a clone designated herein as "DNA58853-1423".

Figure 174 shows the amino acid sequence (SEQ ID NO:256) derived from the coding sequence of SEQ ID NO:255 shown in Figure 173.

5 Figure 175 shows a nucleotide sequence (SEQ ID NO:257) of a native sequence PRO994 (UNQ518) cDNA, wherein SEQ ID NO:257 is a clone designated herein as "DNA58855-1422".

Figure 176 shows the amino acid sequence (SEQ ID NO:258) derived from the coding sequence of SEQ ID NO:257 shown in Figure 175.

Figure 177 shows a nucleotide sequence (SEQ ID NO:259) of a native sequence PRO812 (UNQ517) cDNA, wherein SEQ ID NO:259 is a clone designated herein as "DNA59205-1421".

10 Figure 178 shows the amino acid sequence (SEQ ID NO:260) derived from the coding sequence of SEQ ID NO:259 shown in Figure 177.

Figure 179 shows a nucleotide sequence (SEQ ID NO:261) of a native sequence PRO1069 (UNQ526) cDNA, wherein SEQ ID NO:261 is a clone designated herein as "DNA59211-1450".

15 Figure 180 shows the amino acid sequence (SEQ ID NO:262) derived from the coding sequence of SEQ ID NO:261 shown in Figure 179.

Figure 181 shows a nucleotide sequence (SEQ ID NO:263) of a native sequence PRO1129 (UNQ568) cDNA, wherein SEQ ID NO:263 is a clone designated herein as "DNA59213-1487".

Figure 182 shows the amino acid sequence (SEQ ID NO:264) derived from the coding sequence of SEQ ID NO:263 shown in Figure 181.

20 Figure 183 shows a nucleotide sequence (SEQ ID NO:265) of a native sequence PRO1068 (UNQ525) cDNA, wherein SEQ ID NO:265 is a clone designated herein as "DNA59214-1449".

Figure 184 shows the amino acid sequence (SEQ ID NO:266) derived from the coding sequence of SEQ ID NO:265 shown in Figure 183.

25 Figure 185 shows a nucleotide sequence (SEQ ID NO:267) of a native sequence PRO1066 (UNQ524) cDNA, wherein SEQ ID NO:267 is a clone designated herein as "DNA59215-1425".

Figure 186 shows the amino acid sequence (SEQ ID NO:268) derived from the coding sequence of SEQ ID NO:267 shown in Figure 185.

Figure 187 shows a nucleotide sequence (SEQ ID NO:269) of a native sequence PRO1184 (UNQ598) cDNA, wherein SEQ ID NO:269 is a clone designated herein as "DNA59220-1514".

30 Figure 188 shows the amino acid sequence (SEQ ID NO:270) derived from the coding sequence of SEQ ID NO:269 shown in Figure 187.

Figure 189 shows a nucleotide sequence (SEQ ID NO:271) of a native sequence PRO1360 (UNQ709) cDNA, wherein SEQ ID NO:271 is a clone designated herein as "DNA59488-1603".

35 Figure 190 shows the amino acid sequence (SEQ ID NO:272) derived from the coding sequence of SEQ ID NO:271 shown in Figure 189.

Figure 191 shows a nucleotide sequence (SEQ ID NO:273) of a native sequence PRO1029 (UNQ514) cDNA, wherein SEQ ID NO:273 is a clone designated herein as "DNA59493-1420".

Figure 192 shows the amino acid sequence (SEQ ID NO:274) derived from the coding sequence of SEQ ID NO:273 shown in Figure 191.

Figure 193 shows a nucleotide sequence (SEQ ID NO:275) of a native sequence PRO1139 (UNQ577) cDNA, wherein SEQ ID NO:275 is a clone designated herein as "DNA59497-1496".

5 Figure 194 shows the amino acid sequence (SEQ ID NO:276) derived from the coding sequence of SEQ ID NO:275 shown in Figure 193.

Figure 195 shows a nucleotide sequence (SEQ ID NO:277) of a native sequence PRO1309 (UNQ675) cDNA, wherein SEQ ID NO:277 is a clone designated herein as "DNA59588-1571".

Figure 196 shows the amino acid sequence (SEQ ID NO:278) derived from the coding sequence of SEQ ID NO:277 shown in Figure 195.

10 Figure 197 shows a nucleotide sequence (SEQ ID NO:280) of a native sequence PRO1028 (UNQ513) cDNA, wherein SEQ ID NO:280 is a clone designated herein as "DNA59603-1419".

Figure 198 shows the amino acid sequence (SEQ ID NO:281) derived from the coding sequence of SEQ ID NO:280 shown in Figure 197.

15 Figure 199 shows a nucleotide sequence (SEQ ID NO:282) of a native sequence PRO1027 (UNQ512) cDNA, wherein SEQ ID NO:282 is a clone designated herein as "DNA59605-1418".

Figure 200 shows the amino acid sequence (SEQ ID NO:283) derived from the coding sequence of SEQ ID NO:282 shown in Figure 199.

Figure 201 shows a nucleotide sequence (SEQ ID NO:284) of a native sequence PRO1107 (UNQ550) cDNA, wherein SEQ ID NO:284 is a clone designated herein as "DNA59606-1471".

20 Figure 202 shows the amino acid sequence (SEQ ID NO:285) derived from the coding sequence of SEQ ID NO:284 shown in Figure 201.

Figure 203 shows a nucleotide sequence (SEQ ID NO:286) of a native sequence PRO1140 (UNQ578) cDNA, wherein SEQ ID NO:286 is a clone designated herein as "DNA59607-1497".

25 Figure 204 shows the amino acid sequence (SEQ ID NO:287) derived from the coding sequence of SEQ ID NO:286 shown in Figure 203.

Figure 205 shows a nucleotide sequence (SEQ ID NO:288) of a native sequence PRO1106 (UNQ549) cDNA, wherein SEQ ID NO:288 is a clone designated herein as "DNA59609-1470".

Figure 206 shows the amino acid sequence (SEQ ID NO:289) derived from the coding sequence of SEQ ID NO:288 shown in Figure 205.

30 Figure 207 shows a nucleotide sequence (SEQ ID NO:290) of a native sequence PRO1291 (UNQ659) cDNA, wherein SEQ ID NO:290 is a clone designated herein as "DNA59610-1556".

Figure 208 shows the amino acid sequence (SEQ ID NO:291) derived from the coding sequence of SEQ ID NO:290 shown in Figure 207.

35 Figure 209 shows a nucleotide sequence (SEQ ID NO:292) of a native sequence PRO1105 (UNQ548) cDNA, wherein SEQ ID NO:292 is a clone designated herein as "DNA59612-1466".

Figure 210 shows the amino acid sequence (SEQ ID NO:293) derived from the coding sequence of SEQ ID NO:292 shown in Figure 209.

Figure 211 shows a nucleotide sequence (SEQ ID NO:294) of a native sequence PRO511 (UNQ511) cDNA, wherein SEQ ID NO:294 is a clone designated herein as "DNA59613-1417".

Figure 212 shows the amino acid sequence (SEQ ID NO:295) derived from the coding sequence of SEQ ID NO:294 shown in Figure 211.

Figure 213 shows a nucleotide sequence (SEQ ID NO:296) of a native sequence PRO1104 (UNQ547) cDNA, wherein SEQ ID NO:296 is a clone designated herein as "DNA59616-1465".

Figure 214 shows the amino acid sequence (SEQ ID NO:297) derived from the coding sequence of SEQ ID NO:296 shown in Figure 213.

Figure 215 shows a nucleotide sequence (SEQ ID NO:298) of a native sequence PRO1100 (UNQ546) cDNA, wherein SEQ ID NO:298 is a clone designated herein as "DNA59619-1464".

Figure 216 shows the amino acid sequence (SEQ ID NO:299) derived from the coding sequence of SEQ ID NO:298 shown in Figure 215.

Figure 217 shows a nucleotide sequence (SEQ ID NO:300) of a native sequence PRO836 (UNQ545) cDNA, wherein SEQ ID NO:300 is a clone designated herein as "DNA59620-1463".

Figure 218 shows the amino acid sequence (SEQ ID NO:301) derived from the coding sequence of SEQ ID NO:300 shown in Figure 217.

Figure 219 shows a nucleotide sequence (SEQ ID NO:302) of a native sequence PRO1141 (UNQ579) cDNA, wherein SEQ ID NO:302 is a clone designated herein as "DNA59625-1498".

Figure 220 shows the amino acid sequence (SEQ ID NO:303) derived from the coding sequence of SEQ ID NO:302 shown in Figure 219.

Figure 221 shows a nucleotide sequence designated herein as DNA33128 (SEQ ID NO:304).

Figure 222 shows a nucleotide sequence designated herein as DNA34256 (SEQ ID NO:305).

Figure 223 shows a nucleotide sequence designated herein as DNA47941 (SEQ ID NO:306).

Figure 224 shows a nucleotide sequence designated herein as DNA54389 (SEQ ID NO:307).

Figure 225 shows a nucleotide sequence (SEQ ID NO:308) of a native sequence PRO1132 (UNQ570) cDNA, wherein SEQ ID NO:308 is a clone designated herein as "DNA59767-1489".

Figure 226 shows the amino acid sequence (SEQ ID NO:309) derived from the coding sequence of SEQ ID NO:308 shown in Figure 225.

Figure 227 shows a nucleotide sequence (SEQ ID NO:313) of a native sequence PRO1346 cDNA, wherein SEQ ID NO:313 is a clone designated herein as "DNA59776-1600".

Figure 228 shows the amino acid sequence (SEQ ID NO:314) derived from the coding sequence of SEQ ID NO:313 shown in Figure 227.

Figure 229 shows a nucleotide sequence (SEQ ID NO:318) of a native sequence PRO1131 (UNQ569) cDNA, wherein SEQ ID NO:318 is a clone designated herein as "DNA59777-1480".

Figure 230 shows the amino acid sequence (SEQ ID NO:319) derived from the coding sequence of SEQ ID NO:318 shown in Figure 229.

Figure 231 shows a nucleotide sequence designated herein as DNA43546 (SEQ ID NO:320).

Figure 232 shows a nucleotide sequence (SEQ ID NO:325) of a native sequence PRO1281 (UNQ651) cDNA, wherein SEQ ID NO:325 is a clone designated herein as "DNA59820-1549".

Figure 233 shows the amino acid sequence (SEQ ID NO:326) derived from the coding sequence of SEQ ID NO:325 shown in Figure 232.

Figure 234 shows a nucleotide sequence (SEQ ID NO:333) of a native sequence PRO1064 (UNQ111) cDNA, wherein SEQ ID NO:333 is a clone designated herein as "DNA59827-1426".

Figure 235 shows the amino acid sequence (SEQ ID NO:334) derived from the coding sequence of SEQ ID NO:333 shown in Figure 234.

Figure 236 shows a nucleotide sequence designated herein as DNA45288 (SEQ ID NO:335).

Figure 237 shows a nucleotide sequence (SEQ ID NO:339) of a native sequence PRO1379 (UNQ716) cDNA, wherein SEQ ID NO:339 is a clone designated herein as "DNA59828-1608".

Figure 238 shows the amino acid sequence (SEQ ID NO:340) derived from the coding sequence of SEQ ID NO:339 shown in Figure 237.

Figure 239 shows a nucleotide sequence (SEQ ID NO:344) of a native sequence PRO844 (UNQ544) cDNA, wherein SEQ ID NO:344 is a clone designated herein as "DNA59838-1462".

Figure 240 shows the amino acid sequence (SEQ ID NO:345) derived from the coding sequence of SEQ ID NO:344 shown in Figure 239.

Figure 241 shows a nucleotide sequence (SEQ ID NO:346) of a native sequence PRO848 (UNQ543) cDNA, wherein SEQ ID NO:346 is a clone designated herein as "DNA59839-1461".

Figure 242 shows the amino acid sequence (SEQ ID NO:347) derived from the coding sequence of SEQ ID NO:346 shown in Figure 241.

Figure 243 shows a nucleotide sequence (SEQ ID NO:348) of a native sequence PRO1097 (UNQ542) cDNA, wherein SEQ ID NO:348 is a clone designated herein as "DNA59841-1460".

Figure 244 shows the amino acid sequence (SEQ ID NO:349) derived from the coding sequence of SEQ ID NO:348 shown in Figure 243.

Figure 245 shows a nucleotide sequence (SEQ ID NO:350) of a native sequence PRO1153 (UNQ583) cDNA, wherein SEQ ID NO:350 is a clone designated herein as "DNA59842-1502".

Figure 246 shows the amino acid sequence (SEQ ID NO:351) derived from the coding sequence of SEQ ID NO:350 shown in Figure 245.

Figure 247 shows a nucleotide sequence (SEQ ID NO:352) of a native sequence PRO1154 (UNQ584) cDNA, wherein SEQ ID NO:352 is a clone designated herein as "DNA59846-1503".

Figure 248 shows the amino acid sequence (SEQ ID NO:353) derived from the coding sequence of SEQ ID NO:352 shown in Figure 247.

Figure 249 shows a nucleotide sequence (SEQ ID NO:354) of a native sequence PRO1181 (UNQ595) cDNA, wherein SEQ ID NO:354 is a clone designated herein as "DNA59847-1511".

Figure 250 shows the amino acid sequence (SEQ ID NO:355) derived from the coding sequence of SEQ ID NO:354 shown in Figure 249.

Figure 251 shows a nucleotide sequence (SEQ ID NO:356) of a native sequence PRO1182 (UNQ596) cDNA, wherein SEQ ID NO:356 is a clone designated herein as "DNA59848-1512".

Figure 252 shows the amino acid sequence (SEQ ID NO:357) derived from the coding sequence of SEQ ID NO:356 shown in Figure 251.

Figure 253 shows a nucleotide sequence (SEQ ID NO:358) of a native sequence PRO1155 (UNQ585) cDNA, wherein SEQ ID NO:358 is a clone designated herein as "DNA59849-1504".

Figure 254 shows the amino acid sequence (SEQ ID NO:359) derived from the coding sequence of SEQ ID NO:358 shown in Figure 253.

Figure 255 shows a nucleotide sequence (SEQ ID NO:360) of a native sequence PRO1156 (UNQ586) cDNA, wherein SEQ ID NO:360 is a clone designated herein as "DNA59853-1505".

Figure 256 shows the amino acid sequence (SEQ ID NO:361) derived from the coding sequence of SEQ ID NO:360 shown in Figure 255.

Figure 257 shows a nucleotide sequence (SEQ ID NO:362) of a native sequence PRO1098 (UNQ541) cDNA, wherein SEQ ID NO:362 is a clone designated herein as "DNA59854-1459".

Figure 258 shows the amino acid sequence (SEQ ID NO:363) derived from the coding sequence of SEQ ID NO:362 shown in Figure 257.

Figure 259 shows a nucleotide sequence (SEQ ID NO:364) of a native sequence PRO1127 (UNQ565) cDNA, wherein SEQ ID NO:364 is a clone designated herein as "DNA60283-1484".

Figure 260 shows the amino acid sequence (SEQ ID NO:365) derived from the coding sequence of SEQ ID NO:364 shown in Figure 259.

Figure 261 shows a nucleotide sequence (SEQ ID NO:366) of a native sequence PRO1126 (UNQ564) cDNA, wherein SEQ ID NO:366 is a clone designated herein as "DNA60615-1483".

Figure 262 shows the amino acid sequence (SEQ ID NO:367) derived from the coding sequence of SEQ ID NO:366 shown in Figure 261.

Figure 263 shows a nucleotide sequence (SEQ ID NO:368) of a native sequence PRO1125 (UNQ563) cDNA, wherein SEQ ID NO:368 is a clone designated herein as "DNA60619-1482".

Figure 264 shows the amino acid sequence (SEQ ID NO:369) derived from the coding sequence of SEQ ID NO:368 shown in Figure 263.

Figure 265 shows a nucleotide sequence (SEQ ID NO:370) of a native sequence PRO1186 (UNQ600) cDNA, wherein SEQ ID NO:370 is a clone designated herein as "DNA60621-1516".

Figure 266 shows the amino acid sequence (SEQ ID NO:371) derived from the coding sequence of SEQ ID NO:370 shown in Figure 265.

Figure 267 shows a nucleotide sequence (SEQ ID NO:372) of a native sequence PRO1198 (UNQ611) cDNA, wherein SEQ ID NO:372 is a clone designated herein as "DNA60622-1525".

Figure 268 shows the amino acid sequence (SEQ ID NO:373) derived from the coding sequence of SEQ ID NO:372 shown in Figure 267.

Figure 269 shows a nucleotide sequence (SEQ ID NO:374) of a native sequence PRO1158 (UNQ588) cDNA, wherein SEQ ID NO:374 is a clone designated herein as "DNA60625-1507".

Figure 270 shows the amino acid sequence (SEQ ID NO:375) derived from the coding sequence of SEQ ID NO:374 shown in Figure 269.

Figure 271 shows a nucleotide sequence (SEQ ID NO:376) of a native sequence PRO1159 (UNQ589) cDNA, wherein SEQ ID NO:376 is a clone designated herein as "DNA60627-1508".

5 Figure 272 shows the amino acid sequence (SEQ ID NO:377) derived from the coding sequence of SEQ ID NO:376 shown in Figure 271.

Figure 273 shows a nucleotide sequence (SEQ ID NO:378) of a native sequence PRO1124 (UNQ562) cDNA, wherein SEQ ID NO:378 is a clone designated herein as "DNA60629-1481".

Figure 274 shows the amino acid sequence (SEQ ID NO:379) derived from the coding sequence of SEQ ID NO:378 shown in Figure 273.

10 Figure 275 shows a nucleotide sequence (SEQ ID NO:380) of a native sequence PRO1287 (UNQ656) cDNA, wherein SEQ ID NO:380 is a clone designated herein as "DNA61755-1554".

Figure 276 shows the amino acid sequence (SEQ ID NO:381) derived from the coding sequence of SEQ ID NO:380 shown in Figure 275.

15 Figure 277 shows a nucleotide sequence (SEQ ID NO:386) of a native sequence PRO1312 (UNQ678) cDNA, wherein SEQ ID NO:386 is a clone designated herein as "DNA61873-1574".

Figure 278 shows the amino acid sequence (SEQ ID NO:387) derived from the coding sequence of SEQ ID NO:386 shown in Figure 277.

Figure 279 shows a nucleotide sequence (SEQ ID NO:388) of a native sequence PRO1192 (UNQ606) cDNA, wherein SEQ ID NO:388 is a clone designated herein as "DNA62814-1521".

20 Figure 280 shows the amino acid sequence (SEQ ID NO:389) derived from the coding sequence of SEQ ID NO:388 shown in Figure 279.

Figure 281 shows a nucleotide sequence (SEQ ID NO:393) of a native sequence PRO1160 (UNQ590) cDNA, wherein SEQ ID NO:393 is a clone designated herein as "DNA62872-1509".

25 Figure 282 shows the amino acid sequence (SEQ ID NO:394) derived from the coding sequence of SEQ ID NO:393 shown in Figure 281.

Figure 283 shows a nucleotide sequence (SEQ ID NO:398) of a native sequence PRO1187 (UNQ601) cDNA, wherein SEQ ID NO:398 is a clone designated herein as "DNA62876-1517".

Figure 284 shows the amino acid sequence (SEQ ID NO:399) derived from the coding sequence of SEQ ID NO:398 shown in Figure 283.

30 Figure 285 shows a nucleotide sequence (SEQ ID NO:400) of a native sequence PRO1185 (UNQ599) cDNA, wherein SEQ ID NO:400 is a clone designated herein as "DNA62881-1515".

Figure 286 shows the amino acid sequence (SEQ ID NO:401) derived from the coding sequence of SEQ ID NO:400 shown in Figure 285.

35 Figure 287 shows a nucleotide sequence (SEQ ID NO:402) of a native sequence PRO1345 (UNQ700) cDNA, wherein SEQ ID NO:402 is a clone designated herein as "DNA64852-1589".

Figure 288 shows the amino acid sequence (SEQ ID NO:403) derived from the coding sequence of SEQ ID NO:402 shown in Figure 287.

Figure 289 shows a nucleotide sequence (SEQ ID NO:407) of a native sequence PRO1245 (UNQ629) cDNA, wherein SEQ ID NO:407 is a clone designated herein as "DNA64884-1527".

Figure 290 shows the amino acid sequence (SEQ ID NO:408) derived from the coding sequence of SEQ ID NO:407 shown in Figure 289.

Figure 291 shows a nucleotide sequence (SEQ ID NO:409) of a native sequence PRO1358 (UNQ707) cDNA, wherein SEQ ID NO:409 is a clone designated herein as "DNA64890-1612".

Figure 292 shows the amino acid sequence (SEQ ID NO:410) derived from the coding sequence of SEQ ID NO:409 shown in Figure 291.

Figure 293 shows a nucleotide sequence (SEQ ID NO:411) of a native sequence PRO1195 (UNQ608) cDNA, wherein SEQ ID NO:411 is a clone designated herein as "DNA65412-1523".

Figure 294 shows the amino acid sequence (SEQ ID NO:412) derived from the coding sequence of SEQ ID NO:411 shown in Figure 293.

Figure 295 shows a nucleotide sequence (SEQ ID NO:413) of a native sequence PRO1270 (UNQ640) cDNA, wherein SEQ ID NO:413 is a clone designated herein as "DNA66308-1537".

Figure 296 shows the amino acid sequence (SEQ ID NO:414) derived from the coding sequence of SEQ ID NO:413 shown in Figure 295.

Figure 297 shows a nucleotide sequence (SEQ ID NO:415) of a native sequence PRO1271 (UNQ641) cDNA, wherein SEQ ID NO:415 is a clone designated herein as "DNA66309-1538".

Figure 298 shows the amino acid sequence (SEQ ID NO:416) derived from the coding sequence of SEQ ID NO:415 shown in Figure 297.

Figure 299 shows a nucleotide sequence (SEQ ID NO:417) of a native sequence PRO1375 (UNQ712) cDNA, wherein SEQ ID NO:417 is a clone designated herein as "DNA67004-1614".

Figure 300 shows the amino acid sequence (SEQ ID NO:418) derived from the coding sequence of SEQ ID NO:417 shown in Figure 299.

Figure 301 shows a nucleotide sequence (SEQ ID NO:419) of a native sequence PRO1385 (UNQ720) cDNA, wherein SEQ ID NO:419 is a clone designated herein as "DNA68869-1610".

Figure 302 shows the amino acid sequence (SEQ ID NO:420) derived from the coding sequence of SEQ ID NO:419 shown in Figure 301.

Figure 303 shows a nucleotide sequence (SEQ ID NO:421) of a native sequence PRO1387 (UNQ722) cDNA, wherein SEQ ID NO:421 is a clone designated herein as "DNA68872-1620".

Figure 304 shows the amino acid sequence (SEQ ID NO:422) derived from the coding sequence of SEQ ID NO:421 shown in Figure 303.

Figure 305 shows a nucleotide sequence (SEQ ID NO:423) of a native sequence PRO1384 (UNQ721) cDNA, wherein SEQ ID NO:423 is a clone designated herein as "DNA71159-1617".

Figure 306 shows the amino acid sequence (SEQ ID NO:424) derived from the coding sequence of SEQ ID NO:423 shown in Figure 305.



## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

### I. Definitions

The terms "PRO polypeptide" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (i.e., PRO/number) refers to specific polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" wherein the term "number" is provided an actual numerical designation as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence PRO polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding PRO polypeptide derived from nature. Such native sequence PRO polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of the specific PRO polypeptide (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In various embodiments of the invention, the native sequence PRO281 polypeptide is a mature or full-length native sequence PRO281 polypeptide comprising amino acids 1 to 345 of Figure 2 (SEQ ID NO:2), the native sequence PRO276 is a full-length or mature native sequence PRO276 comprising amino acids 1 through 251 of Figure 4 (SEQ ID NO:6), the native sequence PRO189 is a full-length or mature native sequence PRO189 comprising amino acids 1 through 367 of Figure 6 (SEQ ID NO:8), the native sequence PRO190 polypeptide is a full-length or mature native sequence PRO190 polypeptide comprising amino acids 1 through 424 of Figure 9 (SEQ ID NO:14), the native sequence PRO341 is a mature or full-length native sequence PRO341 comprising amino acids 1 to 458 of Figure 12 (SEQ ID NO:20), the native sequence PRO180 is a full-length or mature native sequence PRO180 comprising amino acids 1 through 266 of Figure 15 (SEQ ID NO:23), the native sequence PRO194 polypeptide is a mature or full-length native sequence PRO194 polypeptide comprising amino acids 1 to 264 of Figure 18 (SEQ ID NO:28), the native sequence PRO203 polypeptide is a mature or full-length native sequence PRO203 polypeptide comprising amino acids 1 to 347 of Figure 20 (SEQ ID NO:30), the native sequence PRO290 is a full-length or mature native sequence PRO290 comprising amino acids 1 through 1003 of Figure 23 (SEQ ID NO:33), the native sequence PRO874 polypeptide comprises amino acids 1 to 321 of Figure 25 (SEQ ID NO:36), the native sequence PRO710 polypeptide is a mature or full-length native sequence PRO710 polypeptide comprising amino acids 1 to 566 of Figure 27 (SEQ ID NO:41), the native sequence PRO1151 is a mature or full-length native sequence PRO1151 comprising amino acids 1 to 259 of Figure 30 (SEQ ID NO:47), the native sequence PRO1282 is a full-length or mature native sequence PRO1282 comprising amino acids 1 or about 24 through 673 of Figure 32 (SEQ ID NO:52), the native sequence PRO358 is a mature or full-length native sequence PRO358 polypeptide comprising amino acids 1 to 811 of Figure 34 (SEQ ID NO:57), the native sequence PRO1310 is a full-length or mature native sequence PRO1310 comprising amino acids 1 through 765 of Figure 36 (SEQ ID NO:62), the native sequence PRO698 polypeptide is a mature or full-length native sequence PRO698 polypeptide comprising amino acids 1 to 510 of Figure 38 (SEQ ID NO:67), the native

sequence PRO732 polypeptide is a mature or full-length native sequence PRO732 polypeptide comprising amino acids 1 to 453 of Figure 41 (SEQ ID NO:73), the native sequence PRO1120 is a full-length or mature native sequence PRO1120 comprising amino acids 1 or about 18 to 867 of Figure 47 (SEQ ID NO:84), the native sequence PRO537 is a mature or full-length native sequence PRO537 comprising amino acids 1 to 115 of Figure 49 (SEQ ID NO:95), the native sequence PRO536 is a mature or full-length native sequence PRO536 comprising amino acids 1 to 313 of Figure 51 (SEQ ID NO:97), the native sequence PRO535 is a mature or full-length native sequence PRO535 comprising amino acids 1 to 201 of Figure 53 (SEQ ID NO:99), the native sequence PRO718 polypeptide is a mature or full-length native sequence PRO718 polypeptide comprising amino acids 1 to 157 of Figure 57 (SEQ ID NO:103), the native sequence PRO872 polypeptide is a mature or full-length native sequence PRO872 polypeptide comprising amino acids 1 to 610 of Figure 64 (SEQ ID NO:113), the native sequence PRO1063 polypeptide is a mature or full-length native sequence PRO1063 polypeptide comprising amino acids 1 to 301 of Figure 66 (SEQ ID NO:115), the native sequence PRO619 is a full-length or mature native sequence PRO619 comprising amino acids 1 or about 21 through 123 of Figure 68 (SEQ ID NO:117), the native sequence PRO943 is a mature or full-length native sequence PRO943 comprising amino acids 1 to 504 of Figure 70 (SEQ ID NO:119), the native sequence PRO1188 is a full-length or mature native sequence PRO1188 comprising amino acids 1 or about 22 to 1184 of Figure 72 (SEQ ID NO:124), the native sequence PRO1133 is a full-length or mature native sequence PRO1133 comprising amino acids 1 or about 19 through 438 of Figure 74 (SEQ ID NO:129), the native sequence PRO784 is a mature or full-length native sequence PRO784 comprising amino acids 16 to 228 of Figure 76 (SEQ ID NO:135), the native sequence PRO783 polypeptide is a mature or full-length native sequence PRO783 polypeptide comprising amino acids 1 to 489 of Figure 79 (SEQ ID NO:138), the native sequence PRO820 is a full-length or mature native sequence PRO820 comprising amino acids 1 or 16 through 124 of Figure 83 (SEQ ID NO:146), the native sequence PRO1080 is a full-length or mature native sequence PRO1080 comprising amino acids 1 or 23 through 358 of Figure 85 (SEQ ID NO:148), the native sequence PRO1079 is a full-length or mature native sequence PRO1079 comprising amino acids 1 or about 30 to 226 of Figure 88 (SEQ ID NO:151), the native sequence PRO793 is a mature or full-length native sequence PRO793 comprising amino acids 1 to 138 of Figure 90 (SEQ ID NO:153), the native sequence PRO1016 is a full-length or mature native sequence PRO1016 comprising amino acids 1 or 19 through 378 of Figure 93 (SEQ ID NO:156), the native sequence PRO1013 polypeptide is a full-length or mature native sequence PRO1013 polypeptide comprising amino acids 1 or 20 through 409 of Figure 95 (SEQ ID NO:158), the native sequence PRO937 polypeptide is a mature or full-length native sequence PRO937 polypeptide comprising amino acids 1 to 556 of Figure 97 (SEQ ID NO:160), the native sequence PRO842 is a full-length or mature native sequence PRO842 comprising amino acids 1 or about 23 to 119 of Figure 99 (SEQ ID NO:165), the native sequence PRO839 is a full-length or mature native sequence PRO839 comprising amino acids 1 or about 24 to 87 of Figure 101 (SEQ ID NO:167), the native sequence PRO1180 polypeptide is a mature or full-length native sequence PRO1180 polypeptide comprising amino acids 1 to 277 of Figure 103 (SEQ ID NO:169), the native sequence PRO1134 is a mature or full-length native sequence PRO1134 comprising amino acids 1 to 371 of Figure 105 (SEQ ID NO:171), the native sequence PRO830 is a mature or full-length native sequence PRO830 comprising amino acids 1 to 87 of Figure 109 (SEQ ID NO:175), the native sequence

PRO1115 is a full-length or mature native sequence PRO1115 comprising amino acids 1 or about 21 to 445 of Figure 111 (SEQ ID NO:177), the native sequence PRO1277 is a full-length or mature native sequence PRO1277 comprising amino acids 1 or about 27 to 678 of Figure 113 (SEQ ID NO:179), the native sequence PRO1135 polypeptide is a mature or full-length native sequence PRO1135 polypeptide comprising amino acids 1 to 541 of Figure 115 (SEQ ID NO:181), the native sequence PRO1114 interferon receptor is a mature or full-length native sequence PRO1114 interferon receptor comprising amino acids 1 to 311 of Figure 118 (SEQ ID NO:184), the native sequence PRO828 polypeptide is a mature or full-length native sequence PRO828 polypeptide comprising amino acids 1 to 187 of Figure 120 (SEQ ID NO:189), the native sequence PRO1009 is a full-length or mature native sequence PRO1009 comprising amino acids 1 or 23 to 615 of Figure 122 (SEQ ID NO:194), the native sequence PRO1007 polypeptide is a full-length or mature native sequence PRO1007 polypeptide comprising amino acids 1 or 31 through 346 of Figure 125 (SEQ ID NO:197), the native sequence PRO1056 is a mature or full-length native sequence PRO1056 comprising amino acids 1 to 120 of Figure 127 (SEQ ID NO:199), the native sequence PRO826 is a mature or full-length native sequence PRO826 comprising amino acids 1 to 99 of Figure 129 (SEQ ID NO:201), the native sequence PRO819 is a mature or full-length native sequence PRO819 comprising amino acids 1 to 52 of Figure 131 (SEQ ID NO:203), the native sequence PRO1006 is a full-length or mature native sequence PRO1006 comprising amino acids 1 or 24 through 392 of Figure 133 (SEQ ID NO:205), the native sequence PRO1112 polypeptide is a full-length or mature native sequence PRO1112 polypeptide comprising amino acids 1 or 14 through 262 of Figure 135 (SEQ ID NO:207), the native sequence PRO1074 polypeptide is a mature or full-length native sequence PRO1074 polypeptide comprising amino acids 1 to 331 of Figure 137 (SEQ ID NO:209), the native sequence PRO1005 is a full-length or mature native sequence PRO1005 comprising amino acids 1 or about 21 to 185 of Figure 139 (SEQ ID NO:211), the native sequence PRO1073 is a full-length or mature native sequence PRO1073 comprising amino acids 1 or about 32 to 299 of Figure 141 (SEQ ID NO:213), the native sequence PRO1152 is a mature or full-length native sequence PRO1152 comprising amino acids 1 to 479 of Figure 144 (SEQ ID NO:216), the native sequence PRO1136 is a mature or full-length native sequence PRO1136 comprising amino acids 1 to 632 of Figure 147 (SEQ ID NO:219), the native sequence PRO813 polypeptide is a mature or full-length native sequence PRO813 polypeptide comprising amino acids 1 to 76 of Figure 149 (SEQ ID NO:221), the native sequence PRO809 is a full-length or mature native sequence PRO809 comprising amino acids 1 or 19 through 265 of Figure 151 (SEQ ID NO:223), the native sequence PRO791 is a full-length or mature native sequence PRO791 comprising amino acids 1 or 26 through 246 of Figure 153 (SEQ ID NO:225), the native sequence PRO1004 is a full-length or mature native sequence PRO1004 comprising amino acids 1 or about 25 through 115 of Figure 155 (SEQ ID NO:227), the native sequence PRO1111 is a full-length or mature native sequence PRO1111 comprising amino acids 1 through 653 of Figure 157 (SEQ ID NO:229), the native sequence PRO1344 is a mature or full-length native sequence PRO1344 comprising amino acids 1 to 720 of Figure 159 (SEQ ID NO:231), the native sequence PRO1109 is a mature or full-length native sequence PRO1109 comprising amino acids 1 to 344 of Figure 161 (SEQ ID NO:236), the native sequence PRO1383 is a mature or full-length native sequence PRO1383 comprising amino acids 1 to 423 of Figure 163 (SEQ ID NO:241), the native sequence PRO1003 polypeptide is a mature or full-length native sequence PRO1003 polypeptide comprising amino acids

1 to 84 of Figure 165 (SEQ ID NO:246), the native sequence PRO1108 polypeptide is a mature or full-length native sequence PRO1108 polypeptide comprising amino acids 1 to 456 of Figure 167 (SEQ ID NO:248), the native sequence PRO1137 polypeptide is a mature or full-length native sequence PRO1137 polypeptide comprising amino acids 1 to 240 of Figure 169 (SEQ ID NO:250), the native sequence PRO1138 polypeptide is a mature or full-length native sequence PRO1138 polypeptide comprising amino acids 1 to 335 of Figure 171 (SEQ ID NO:253), the native sequence PRO1054 is a mature or full-length native sequence PRO1054 comprising amino acids 1 to 180 of Figure 174 (SEQ ID NO:256), the native sequence PRO994 is a mature or full-length native sequence PRO994 comprising amino acids 1 to 229 of Figure 176 (SEQ ID NO:258), the native sequence PRO812 is a mature or full-length native sequence PRO812 comprising amino acids 1 to 83 of Figure 178 (SEQ ID NO:260), the native sequence PRO1069 polypeptide is a mature or full-length native sequence PRO1069 polypeptide comprising amino acids 1 to 89 of Figure 180 (SEQ ID NO:262), the native sequence PRO1129 polypeptide is a mature or full-length native sequence PRO1129 polypeptide comprising amino acids 1 to 524 of Figure 182 (SEQ ID NO:264), the native sequence PRO1068 is a full-length or mature native sequence PRO1068 comprising amino acids 1 or about 21 to 124 of Figure 184 (SEQ ID NO:266), the native sequence PRO1066 polypeptide is a mature or full-length native sequence PRO1066 polypeptide comprising amino acids 1 to 117 of Figure 186 (SEQ ID NO:268), the native sequence PRO1184 polypeptide is a full-length or mature native sequence PRO1184 polypeptide comprising amino acids 1 or 39 through 142 of Figure 188 (SEQ ID NO:270), the native sequence PRO1360 is a full-length or mature native sequence PRO1360 comprising amino acids 1 or about 30 through 285 of Figure 190 (SEQ ID NO:272), the native sequence PRO1029 is a mature or full-length native sequence PRO1029 comprising amino acids 1 to 86 of Figure 192 (SEQ ID NO:274), the native sequence PRO1139 is a mature or full-length native sequence PRO1139 polypeptide comprising amino acids 1 to 131 or 29-131 of Figure 194 (SEQ ID NO:276), the native sequence PRO1309 is a full-length or mature native sequence PRO1309 comprising amino acids 1 or about 35 through 522 of Figure 196 (SEQ ID NO:278), the native sequence PRO1028 polypeptide is a full-length or mature native sequence PRO1028 polypeptide comprising amino acids 1 or 20 through 197 of Figure 198 (SEQ ID NO:281), the native sequence PRO1027 is a full-length or mature native sequence PRO1027 comprising amino acids 1 or 34 through 77 of Figure 200 (SEQ ID NO:283), the native sequence PRO1107 polypeptide is a full-length or mature native sequence PRO1107 polypeptide comprising amino acids 1 or 23 through 477 of Figure 202 (SEQ ID NO:285), the native sequence PRO1140 polypeptide is a mature or full-length native sequence PRO1140 polypeptide comprising amino acids 1 to 255 of Figure 204 (SEQ ID NO:287), the native sequence PRO1106 polypeptide is a full-length or mature native sequence PRO1106 polypeptide comprising amino acids 1 or 17 through 469 of Figure 206 (SEQ ID NO:289), the native sequence PRO1291 is a mature or full-length native sequence PRO1291 comprising amino acids 1 to 282 of Figure 208 (SEQ ID NO:291), the native sequence PRO1105 polypeptide is a full-length or mature native sequence PRO1105 polypeptide comprising amino acids 1 or 20 through 180 of Figure 210 (SEQ ID NO:293), the native sequence PRO1026 is a full-length or mature native sequence PRO1026 comprising amino acids 1 or 26 through 237 of Figure 212 (SEQ ID NO:295), the native sequence PRO1104 is a full-length or mature native sequence PRO1104 comprising amino acids 1 or about 23 through 341 of Figure 214 (SEQ ID NO:297), the native sequence PRO1100 is a full-length or mature native

sequence PRO1100 comprising amino acids 1 or 21 through 320 of Figure 216 (SEQ ID NO:299), the native sequence PRO836 is a full-length or mature native sequence PRO836 comprising amino acids 1 or 30 through 461 of Figure 218 (SEQ ID NO:301), the native sequence PRO1141 is a mature or full-length native sequence PRO1141 comprising amino acids 1 to 247 of Figure 220 (SEQ ID NO:303), the native sequence PRO1132 is a full-length or mature native sequence PRO1132 comprising amino acids 1 or about 23 through 293 of Figure 226 (SEQ ID NO:309), the native sequence NL7 is a mature or full-length native sequence NL7 comprising amino acids from about position 51 to about position 461 of Figure 228 (SEQ ID NO:314), the native sequence PRO1131 is a full-length or mature native sequence PRO1131 comprising amino acids 1 through 280 of Figure 230 (SEQ ID NO:319), the native sequence PRO1281 is a full-length or mature native sequence PRO1281 comprising amino acids 1 or about 16 to 775 of Figure 233 (SEQ ID NO:326), the native sequence PRO1064 is a mature or full-length native sequence PRO1064 comprising amino acids 1 to 153 of Figure 235 (SEQ ID NO:334), the native sequence PRO1379 is a full-length or mature native sequence PRO1379 comprising amino acids 1 or about 18 to 574 of Figure 238 (SEQ ID NO:340), the native sequence PRO844 is a full-length or mature native sequence PRO844 comprising amino acids 1 or 20 through 111 of Figure 240 (SEQ ID NO:344), the native sequence PRO848 is a full-length or mature native sequence PRO848 comprising amino acids 1 or 36 through 600 of Figure 242 (SEQ ID NO:347), the native sequence PRO1097 is a full-length or mature native sequence PRO1097 comprising amino acids 1 or 21 through 91 of Figure 244 (SEQ ID NO:349), the native sequence PRO1153 is a mature or full-length native sequence PRO1153 comprising amino acids 1 to 197 of Figure 246 (SEQ ID NO:351), the native sequence PRO1154 is a full-length or mature native sequence PRO1154 comprising amino acids 1 or 35 to 941 of Figure 248 (SEQ ID NO:353), the native sequence PRO1181 is a mature or full-length native sequence PRO1181 comprising amino acids 1 to 437 of Figure 250 (SEQ ID NO:355), the native sequence PRO1182 is a mature or full-length native sequence PRO1182 comprising amino acids 1 to 271 of Figure 252 (SEQ ID NO:357), the native sequence PRO1155 is a full-length native or mature sequence PRO1155 comprising amino acids 1 or 19 through 135 of Figure 254 (SEQ ID NO:359), the native sequence PRO1156 is a full-length or mature native sequence PRO1156 comprising amino acids 1 or about 23 to 159 of Figure 256 (SEQ ID NO:361), the native sequence PRO1098 is a full-length or mature native sequence PRO1098 comprising amino acids 1 or 20 through 78 of Figure 258 (SEQ ID NO:363), the native sequence PRO1127 is a full-length or mature native sequence PRO1127 comprising amino acids 1 or about 30 through 67 of Figure 260 (SEQ ID NO:365), the native sequence PRO1126 is a mature or full-length native sequence PRO1126 comprising amino acids 1 to 402 of Figure 262 (SEQ ID NO:367), the native sequence PRO1125 is a mature or full-length native sequence PRO1125 comprising amino acids 26 to 447 of Figure 264 (SEQ ID NO:369), the native sequence PRO1186 is a full-length or mature native sequence PRO1186 comprising amino acids 1 or about 20 through 105 of Figure 266 (SEQ ID NO:371), the native sequence PRO1198 is a full-length or mature native sequence PRO1198 comprising amino acids 1 or about 35 to 229 of Figure 268 (SEQ ID NO:373), the native sequence PRO1158 is a full-length or mature native sequence PRO1158 comprising amino acids 1 or about 20 to 123 of Figure 270 (SEQ ID NO:375), the native sequence PRO1159 is a mature or full-length native sequence PRO1159 comprising amino acids 1 to 90 of Figure 272 (SEQ ID NO:377), the native sequence PRO1124 is a mature or full-length native sequence PRO1124 comprising amino acids 22 through 919

of Figure 274 (SEQ ID NO:379), the native sequence PRO1287 is a mature or full-length native sequence PRO1287 comprising amino acids 1 to 532 of Figure 276 (SEQ ID NO:381), the native sequence PRO1312 is a full-length or mature native sequence PRO1312 comprising amino acids 1 or about 15 to 212 of Figure 278 (SEQ ID NO:387), the native sequence PRO1192 is a full-length or mature native sequence PRO1192 comprising amino acids 1 or about 22 to 215 of Figure 280 (SEQ ID NO:389), the native sequence PRO1160 is a mature or full-length native sequence PRO1160 comprising amino acids 1 to 90 of Figure 282 (SEQ ID NO:394), the native sequence PRO1187 is a full-length or mature native sequence PRO1187 comprising amino acids 1 or about 18 through 120 of Figure 284 (SEQ ID NO:399), the native sequence PRO1185 is a full-length or mature native sequence PRO1185 comprising amino acids 1 or about 22 through 198 of Figure 286 (SEQ ID NO:401), the native sequence PRO1345 is a mature or full-length native sequence PRO1345 comprising amino acids 1 to 206 of Figure 288 (SEQ ID NO:403), the native sequence PRO1245 is a full-length or mature native sequence PRO1245 comprising amino acids 1 or about 19 to 104 of Figure 290 (SEQ ID NO:408), the native sequence PRO1358 is a full-length or mature native sequence PRO1358 comprising amino acids 1 or about 19 through 444 of Figure 292 (SEQ ID NO:410), the native sequence PRO1195 is a full-length or mature native sequence PRO1195 comprising amino acids 1 or about 23 through 151 of Figure 294 (SEQ ID NO:412), the native sequence PRO1270 is a mature or full-length native sequence PRO1270 comprising amino acids 1 to 313 of Figure 296 (SEQ ID NO:414), the native sequence PRO1271 is a mature or full-length native sequence PRO1271 comprising amino acids 1 to 208 of Figure 298 (SEQ ID NO:416), the native sequence PRO1375 is a full-length or mature native sequence PRO1375 comprising amino acids 1 through 198 of Figure 300 (SEQ ID NO:418), the native sequence PRO1385 is a mature or full-length native sequence PRO1385 comprising amino acids 1 to 128 of Figure 302 (SEQ ID NO:420), the native sequence PRO1387 is a mature or full-length native sequence PRO1387 comprising amino acids 1 to 394 of Figure 304 (SEQ ID NO:422) and the native sequence PRO1384 is a full-length or mature native sequence PRO1384 comprising amino acids 1 to 229 of Figure 306 (SEQ ID NO:424). Start and stop codons are shown in bold font and underlined in the figures.

The PRO polypeptide "extracellular domain" or "ECD" refers to a form of the PRO polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a PRO polypeptide ECD will have less than 1 % of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5 % of such domains. It will be understood that any transmembrane domains identified for the PRO polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified. Optionally, therefore, an extracellular domain of a PRO polypeptide may contain from about 5 or fewer amino acids on either or the transmembrane domain as initially identified.

"PRO polypeptide variant" means an active PRO polypeptide as defined above or below having at least about 80% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, PRO

polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, preferably at least about 81% amino acid sequence identity, more preferably at least about 82% amino acid sequence identity, more preferably at least about 83% amino acid sequence identity, more preferably at least about 84% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, more preferably at least about 86% amino acid sequence identity, more preferably at least about 87% amino acid sequence identity, more preferably at least about 88% amino acid sequence identity, more preferably at least about 89% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, more preferably at least about 91% amino acid sequence identity, more preferably at least about 92% amino acid sequence identity, more preferably at least about 93% amino acid sequence identity, more preferably at least about 94% amino acid sequence identity, more preferably at least about 95% amino acid sequence identity, more preferably at least about 96% amino acid sequence identity, more preferably at least about 97% amino acid sequence identity, more preferably at least about 98% amino acid sequence identity and most preferably at least about 99% amino acid sequence identity with the amino acid sequence of the full-length native amino acid sequence as disclosed herein. Ordinarily, PRO variant polypeptides are at least about 10 amino acids in length, often at least about 20 amino acids in length, more often at least about 30 amino acids in length, more often at least about 40 amino acids in length, more often at least about 50 amino acids in length, more often at least about 60 amino acids in length, more often at least about 70 amino acids in length, more often at least about 80 amino acids in length, more often at least about 90 amino acids in length, more often at least about 100 amino acids in length, more often at least about 150 amino acids in length, more often at least about 200 amino acids in length, more often at least about 300 amino acids in length, or more.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific PRO polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the WU-BLAST-2 computer program (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. For purposes herein, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the PRO polypeptide of interest having a sequence derived from the native PRO polypeptide and the comparison amino acid sequence of interest (i.e., the sequence against which the PRO polypeptide of interest is being compared which may be

a PRO variant polypeptide) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest.

"PRO variant polynucleotide" or "PRO variant nucleic acid sequence" means a nucleic acid molecule which encodes an active PRO polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, a PRO variant polynucleotide will have at least about 80% nucleic acid sequence identity, more preferably at least about 81% nucleic acid sequence identity, more preferably at least about 82% nucleic acid sequence identity, more preferably at least about 83% nucleic acid sequence identity, more preferably at least about 84% nucleic acid sequence identity, more preferably at least about 85% nucleic acid sequence identity, more preferably at least about 86% nucleic acid sequence identity, more preferably at least about 87% nucleic acid sequence identity, more preferably at least about 88% nucleic acid sequence identity, more preferably at least about 89% nucleic acid sequence identity, more preferably at least about 90% nucleic acid sequence identity, more preferably at least about 91% nucleic acid sequence identity, more preferably at least about 92% nucleic acid sequence identity, more preferably at least about 93% nucleic acid sequence identity, more preferably at least about 94% nucleic acid sequence identity, more preferably at least about 95% nucleic acid sequence identity, more preferably at least about 96% nucleic acid sequence identity, more preferably at least about 97% nucleic acid sequence identity, more preferably at least about 98% nucleic acid sequence identity and yet more preferably at least about 99% nucleic acid sequence identity with the nucleic acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Variants do not encompass the native nucleotide sequence.

Ordinarily, PRO variant polynucleotides are at least about 30 nucleotides in length, often at least about 60 nucleotides in length, more often at least about 90 nucleotides in length, more often at least about 120 nucleotides in length, more often at least about 150 nucleotides in length, more often at least about 180 nucleotides in length, more often at least about 210 nucleotides in length, more often at least about 240 nucleotides in length, more often at least about 270 nucleotides in length, more often at least about 300 nucleotides in length, more often at least about 450 nucleotides in length, more often at least about 600 nucleotides in length, more often at least about 900 nucleotides in length, or more.

"Percent (%) nucleic acid sequence identity" with respect to PRO-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO nucleic acid sequence of interest, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR)



software. For purposes herein, however, % nucleic acid sequence identity values are generated using the WU-BLAST-2 computer program (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. For purposes herein, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest having a sequence derived from the native sequence PRO polypeptide-encoding nucleic acid and the comparison nucleic acid molecule of interest (i.e., the sequence against which the PRO polypeptide-encoding nucleic acid molecule of interest is being compared which may be a variant PRO polynucleotide) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO polypeptide-encoding nucleic acid molecule of interest.

In other embodiments, PRO variant polynucleotides are nucleic acid molecules that encode an active PRO polypeptide and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding a full-length PRO polypeptide as disclosed herein. PRO variant polypeptides may be those that are encoded by a PRO variant polynucleotide.

The term "positives", in the context of sequence comparison performed as described above, includes residues in the sequences compared that are not identical but have similar properties (e.g. as a result of conservative substitutions, see Table 1 below). For purposes herein, the % value of positives is determined by dividing (a) the number of amino acid residues scoring a positive value between the PRO polypeptide amino acid sequence of interest having a sequence derived from the native PRO polypeptide sequence and the comparison amino acid sequence of interest (i.e., the amino acid sequence against which the PRO polypeptide sequence is being compared) as determined in the BLOSUM62 matrix of WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the PRO polypeptide nucleic acid. An isolated PRO polypeptide nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated PRO polypeptide nucleic acid molecules therefore are distinguished from the specific PRO polypeptide nucleic acid molecule as it exists in natural cells. However,

an isolated PRO polypeptide nucleic acid molecule includes PRO polypeptide nucleic acid molecules contained in cells that ordinarily express the PRO polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-PRO monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-PRO antibody compositions with polypeptidic specificity, single chain anti-PRO antibodies, and fragments of anti-PRO antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M

sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

"Active" or "activity" for the purposes herein refers to form(s) of a PRO polypeptide which retain a biological and/or an immunological activity of native or naturally-occurring PRO, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring PRO other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically

include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO polypeptides, peptides, small organic molecules, etc. Methods for identifying agonists or antagonists of a PRO polypeptide may comprise contacting a PRO polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the PRO polypeptide.

5 "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to  
10 an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats,  
15 rabbits, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often  
20 the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including  
25 glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv  
30 fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen-combining sites  
35 and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific  
5 for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge  
10 region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant  
15 domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

"Single-chain Fv" or "sFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).  
20

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH - VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and  
25 Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1)  
35 to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or

nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself  
5 (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and  
10 silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a PRO polypeptide or antibody thereto) to a mammal. The  
15 components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

## II. Compositions and Methods of the Invention

20 The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO polypeptides. In particular, cDNAs encoding various PRO polypeptides have been identified and isolated, as disclosed in further detail in the Examples below. It is noted that proteins produced in separate expression rounds may be given different PRO numbers but the UNQ number is unique for any given DNA and the encoded protein, and will not be changed. However, for sake of  
25 simplicity, in the present specification the protein encoded by the full length native nucleic acid molecules disclosed herein as well as all further native homologues and variants included in the foregoing definition of PRO, will be referred to as "PRO/number", regardless of their origin or mode of preparation.

As disclosed in the Examples below, various cDNA clones have been deposited with the ATCC. The actual nucleotide sequences of those clones can readily be determined by the skilled artisan by sequencing of the  
30 deposited clone using routine methods in the art. The predicted amino acid sequence can be determined from the nucleotide sequence using routine skill. For the PRO polypeptides and encoding nucleic acids described herein, Applicants have identified what is believed to be the reading frame best identifiable with the sequence information available at the time.

### 35 1. Full-length PRO281 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO281 (UNQ244). In particular, cDNA encoding a PRO281

the L6 cell surface tumor cell-associated antigen, designated herein as PRO994 polypeptides.

74. **PRO812**

Steroid binding proteins play important roles in numerous physiological processes associated with steroid function. Specifically, one steroid binding protein-associated polypeptide that has been well characterized is component 1 of the prostatic binding protein. Component 1 of the prostatic binding protein has been shown to be specific for subunit F of the prostatic binding protein, the major secretory glycoprotein of the rat ventral prostate (Peeters et al., Eur. J. Biochem. 123:55-62 (1982) and Liao et al., J. Biol. Chem. 257:122-125 (1982)). The amino acid sequence of component 1 of the prostatic binding protein has been determined, wherein the sequence is highly rich in glutamic acid residues and is overall highly acidic. This protein plays an important role in the response of the prostate gland to steroid hormones. We herein describe the identification and characterization of novel polypeptides having homology to prostatic steroid-binding protein c1, designated herein as PRO812 polypeptides.

75. **PRO1069**

Of particular interest is the identification of new membrane-bound proteins involved in ion conductance such as channel inhibitory factor (CHIF) and MAT-8, which have recently been reported (see Wald et al., Am. J. Physiol. 272(5 pt 2): F617-F623 (1997); Capurro et al., Am. J. Physiol. 271(3 pt 1): C753-C762 (1996); Wald et al., Am. J. Physiol. 271(2 pt 2): F322-F329 (1996); and Morrison et al., J. Biol. Chem. 270(5):2176-2182 (1995)).

Described herein is the identification and characterization of novel polypeptides having homology to CHIF and MAT-8 polypeptides, designated herein as PRO1069 polypeptides.

76. **PRO1129**

Cytochromes P-450 are a superfamily of hemoproteins which represent the main pathway for drug and chemical oxidation (Horsmans, Acta Gastroenterol. Belg. 60:2-10 (1997)). This superfamily is divided into families, subfamilies and/or single enzymes. Recent reports have provided a great deal of information concerning the cytochrome P-450 isozymes and increased awareness of life threatening interactions with such commonly prescribed drugs as cisapride and some antihistamines (Michalets, Pharmacotherapy 18:84-112 (1998) and Singer et al., J. Am. Acad. Dermatol. 37:765-771 (1997)). Given this information, there is significant interest in identifying novel members of the cytochrome P-450 family of proteins. We herein describe the identification and characterization of novel polypeptides having homology to cytochrome P-450 proteins, designated herein as PRO1129 polypeptides.

77. **PRO1068**

Urotensins are neurosecretory proteins that are of interest because of their potential roles in a variety of physiological processes including smooth muscle contraction (Yano et al. Gen. Comp. Endocrinol. 96(3): 412-413 (1994)), regulation of arterial blood pressure and heart rate (Le Mevel et al. Am. J. Physiol. 271(5 Pt 2):

of receptor proteins is of interest (see Houchins JP, *et al. J. Exp. Med.* (1991) 173(4):1017-1020).

### SUMMARY OF THE INVENTION

#### 1. PRO281

5 A cDNA clone (DNA16422-1209) has been identified, having homology to nucleic acid encoding testis enhanced gene transcript (TEGT) protein that encodes a novel polypeptide, designated in the present application as "PRO281".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO281 polypeptide.

10 In one aspect, the isolated nucleic acid comprises DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding a PRO281 polypeptide having the sequence of amino acid residues from about 1 or about 15 to about 345, inclusive of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA molecule of (a).

15 In another aspect, the invention concerns an isolated nucleic acid molecule encoding a PRO281 polypeptide comprising DNA hybridizing to the complement of the nucleic acid between about nucleotides 80 or about 122 and about 1114, inclusive, of Figure 1 (SEQ ID NO:1). Preferably, hybridization occurs under stringent hybridization and wash conditions.

20 In a further aspect, the invention concerns an isolated nucleic acid molecule comprising DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. 209929 (DNA16422-1209) or (b) the complement of the nucleic acid molecule of (a). In a preferred embodiment, the nucleic acid comprises a DNA encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. 209929 (DNA16422-1209).

25 In still a further aspect, the invention concerns an isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues 1 or about 15 to about 345, inclusive of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA of (a).

30 In a further aspect, the invention concerns an isolated nucleic acid molecule having at least 10 nucleotides and produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO281 polypeptide having the sequence of amino acid residues from 1 or about 15 to about 345, inclusive of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA molecule of (a), and, if the DNA molecule has at least about an 80% sequence identity, preferably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), isolating the test DNA molecule.

35



In a still further embodiment, the invention concerns a composition comprising a PRO994 polypeptide, or an agonist or antagonist as hereinabove defined, in combination with a pharmaceutically acceptable carrier.

74. **PRO812**

5 A cDNA clone (DNA59205-1421) has been identified, having homology to nucleic acid encoding prostatic steroid-binding protein c1 that encodes a novel polypeptide, designated in the present application as "PRO812".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO812 polypeptide.

10 In one aspect, the isolated nucleic acid comprises DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding a PRO812 polypeptide having the sequence of amino acid residues from about 1 or about 16 to about 83, inclusive of Figure 178 (SEQ ID NO:260), or (b) the complement of the DNA molecule of (a).

15 In another aspect, the invention concerns an isolated nucleic acid molecule encoding a PRO812 polypeptide comprising DNA hybridizing to the complement of the nucleic acid between about nucleotides 55 or about 100 and about 303, inclusive, of Figure 177 (SEQ ID NO:259). Preferably, hybridization occurs under stringent hybridization and wash conditions.

20 In a further aspect, the invention concerns an isolated nucleic acid molecule comprising DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. 203009 (DNA59205-1421) or (b) the complement of the nucleic acid molecule of (a). In a preferred embodiment, the nucleic acid comprises a DNA encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. 203009 (DNA59205-1421).

25 In still a further aspect, the invention concerns an isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues 1 or about 16 to about 83, inclusive of Figure 178 (SEQ ID NO:260), or (b) the complement of the DNA of (a).

30 In a further aspect, the invention concerns an isolated nucleic acid molecule having at least 100 nucleotides and produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO812 polypeptide having the sequence of amino acid residues from 1 or about 16 to about 83, inclusive of Figure 178 (SEQ ID NO:260), or (b) the complement of the DNA molecule of (a), and, if the DNA molecule has at least about an 80 % sequence identity, preferably at least about an 85% sequence  
35 identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), isolating the test DNA molecule.

In a specific aspect, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO812 polypeptide, with or without the N-terminal signal sequence and/or the initiating methionine, or is complementary to such encoding nucleic acid molecule. The signal peptide has been tentatively identified as extending from about amino acid position 1 to about amino acid position 15 in the sequence of Figure 178 (SEQ ID NO:260).

5 In another aspect, the invention concerns an isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 1 or about 16 to about 83, inclusive of Figure 178 (SEQ ID NO:260), or (b) the complement of the DNA of (a).

10 Another embodiment is directed to fragments of a PRO812 polypeptide coding sequence that may find use as hybridization probes. Such nucleic acid fragments are from about 20 to about 80 nucleotides in length, preferably from about 20 to about 60 nucleotides in length, more preferably from about 20 to about 50 nucleotides in length and most preferably from about 20 to about 40 nucleotides in length and may be derived from the nucleotide sequence shown in Figure 177 (SEQ ID NO:259).

15 In another embodiment, the invention provides isolated PRO812 polypeptide encoded by any of the isolated nucleic acid sequences hereinabove identified.

In a specific aspect, the invention provides isolated native sequence PRO812 polypeptide, which in certain embodiments, includes an amino acid sequence comprising residues 1 or about 16 to about 83 of Figure 178 (SEQ ID NO:260).

20 In another aspect, the invention concerns an isolated PRO812 polypeptide, comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues 1 or about 16 to about 83, inclusive of Figure 178 (SEQ ID NO:260).

25 In a further aspect, the invention concerns an isolated PRO812 polypeptide, comprising an amino acid sequence scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 1 or about 16 to about 83, inclusive of Figure 178 (SEQ ID NO:260).

30 In yet another aspect, the invention concerns an isolated PRO812 polypeptide, comprising the sequence of amino acid residues 1 or about 16 to about 83, inclusive of Figure 178 (SEQ ID NO:260), or a fragment thereof sufficient to provide a binding site for an anti-PRO812 antibody. Preferably, the PRO812 fragment retains a qualitative biological activity of a native PRO812 polypeptide.

35 In a still further aspect, the invention provides a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO812 polypeptide having the sequence of amino acid residues from about 1 or about 16 to about 83, inclusive of Figure 178 (SEQ ID NO:260), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has at least about an 80% sequence identity, preferably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), (ii) culturing a host

cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO812 polypeptide. In a particular embodiment, the agonist or antagonist is an anti-PRO812 antibody.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists of a native PRO812 polypeptide by contacting the native PRO812 polypeptide with a candidate molecule and monitoring a biological activity mediated by said polypeptide.

In a still further embodiment, the invention concerns a composition comprising a PRO812 polypeptide, or an agonist or antagonist as hereinabove defined, in combination with a pharmaceutically acceptable carrier.

10 75. PRO1069

Applicants have identified a cDNA clone, DNA59211-1450, that encodes a novel polypeptide having homology to CHIF wherein the polypeptide is designated in the present application as "PRO1069".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO1069 polypeptide.

15 In one aspect, the isolated nucleic acid comprises DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding a PRO1069 polypeptide having the sequence of amino acid residues from 1 or about 17 to about 89, inclusive of Figure 180 (SEQ ID NO:262), or (b) the complement of the DNA molecule of (a).

20 In another aspect, the invention concerns an isolated nucleic acid molecule encoding a PRO1069 polypeptide comprising DNA that hybridizes to the complement of the nucleic acid sequence having about residues 197 or about 245 to about 463, inclusive of Figure 179 (SEQ ID NO:261). Preferably, hybridization occurs under stringent hybridization and wash conditions.

25 In a further aspect, the invention concerns an isolated nucleic acid molecule comprising DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. 209960 (DNA59211-1450), which was deposited on June 9, 1998. In a preferred embodiment, the nucleic acid comprises a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA in  
30 ATCC Deposit No. 209960 (DNA59211-1450).

In a still further aspect, the invention concerns an isolated nucleic acid molecule comprising DNA encoding a polypeptide having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues 1 or about 17 to about 89, inclusive of Figure 180 (SEQ ID  
35 NO:262).

In a specific aspect, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO1069 extracellular domain (ECD), with or without the N-terminal signal sequence and/or the initiating

**72. Full-length PRO1054 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO1054. In particular, cDNA encoding a PRO1054 polypeptide has been identified and isolated, as disclosed in further detail in the Examples below.

5 Using the WU-BLAST2 sequence alignment computer program, it has been found that a full-length native sequence PRO1054 (shown in Figure 174 and SEQ ID NO:256) has amino acid sequence identity with one or more of the major urinary proteins. Accordingly, it is presently believed that PRO1054 disclosed in the present application is a newly identified member of the MUP family and may possess activity typical of that family.

**10 73. Full-length PRO994 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO994. In particular, cDNA encoding a PRO994 polypeptide has been identified and isolated, as disclosed in further detail in the Examples below.

15 Using the WU-BLAST2 sequence alignment computer program, it has been found that a full-length native sequence PRO994 (shown in Figure 176 and SEQ ID NO:258) has amino acid sequence identity with the tumor-associated antigen L6. Accordingly, it is presently believed that PRO994 disclosed in the present application is a newly identified L6 antigen homolog.

**20 74. Full-length PRO812 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO812. In particular, cDNA encoding a PRO812 polypeptide has been identified and isolated, as disclosed in further detail in the Examples below.

25 Using the WU-BLAST2 sequence alignment computer program, it has been found that a full-length native sequence PRO812 (shown in Figure 178 and SEQ ID NO:260) has amino acid sequence identity with the prostatic steroid-binding c1 protein. Accordingly, it is presently believed that PRO812 disclosed in the present application is a newly identified prostatic steroid-binding c1 protein homolog.

**75. Full-length PRO1069 Polypeptides**

30 The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO1069. In particular, Applicants have identified and isolated cDNA encoding a PRO1069 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, it was found that the PRO1069 polypeptide has sequence identity with CHIF. Accordingly, it is presently believed that PRO1069 polypeptide disclosed in the present application is a newly identified CHIF polypeptide and is involved in ion conductance or regulation of ion conductance.

35

Using the WU-BLAST2 sequence alignment computer program, it has been found that a full-length native sequence PRO1387 (shown in Figure 304 and SEQ ID NO:422) has amino acid sequence identity with the myelin p0 protein precursor (MYP0\_HETFR). Accordingly, it is presently believed that PRO1387 disclosed in the present application is a newly identified member of the myelin protein family and may possess activity typical of that family.

5

### 135. Full-length PRO1384 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO1384. In particular, cDNA encoding a PRO1384 polypeptide has been identified and isolated, as disclosed in further detail in the Examples below.

10

Using WU-BLAST2 sequence alignment computer programs, it has been found that a full-length native sequence PRO1384 (shown in Figure 306 and SEQ ID NO:424) has amino acid sequence identity with NKG2-D (AF054819\_1; Dayhoff database, version 35.45 SwissProt 35). Accordingly, it is presently believed that PRO1384 disclosed in the present application is a newly identified member of the NKG2 family and may possess MHC activation/inactivation activities typical of the NKG2 family.

15

### B. PRO Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated that PRO variants can be prepared. PRO variants can be prepared by introducing appropriate nucleotide changes into the PRO DNA, and/or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

20

Variations in the native full-length sequence PRO or in various domains of the PRO described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO that results in a change in the amino acid sequence of the PRO as compared with the native sequence PRO. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

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PRO polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native protein.

Certain fragments lack amino acid residues that are not essential for a desired biological activity of the PRO polypeptide.

5 PRO fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating PRO fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the  
10 desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, PRO polypeptide fragments share at least one biological and/or immunological activity with the native PRO polypeptide disclosed herein.

In particular embodiments, conservative substitutions of interest are shown in Table 1 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 1

	<u>Original Residue</u>	<u>Exemplary Substitutions</u>	<u>Preferred Substitutions</u>
5	Ala (A)	val; leu; ile	val
	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
	Asp (D)	glu	glu
	Cys (C)	ser	ser
10	Gln (Q)	asn	asn
	Glu (E)	asp	asp
	Gly (G)	pro; ala	ala
	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe;	
15		norleucine	leu
	Leu (L)	norleucine; ile; val;	
		met; ala; phe	ile
	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
20	Phe (F)	leu; val; ile; ala; tyr	leu
	Pro (P)	ala	ala
	Ser (S)	thr	thr
	Thr (T)	ser	ser
	Trp (W)	tyr; phe	tyr
25	Tyr (Y)	trp; phe; thr; ser	phe
	Val (V)	ile; leu; met; phe;	
		ala; norleucine	leu

Substantial modifications in function or immunological identity of the PRO polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10:6487 (1987)], cassette mutagenesis [Wells et al., Gene, 34:315 (1985)], restriction selection mutagenesis [Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO variant

DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, Science, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

10

### C. Modifications of PRO

Covalent modifications of PRO are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the PRO. Derivatization with bifunctional agents is useful, for instance, for crosslinking PRO to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimide.

20

Other modifications include deamidation of glutamyl and asparaginy residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  $\alpha$ -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

25

Another type of covalent modification of the PRO polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

30

Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO (for O-linked glycosylation sites). The PRO amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

35



Another means of increasing the number of carbohydrate moieties on the PRO polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., Arch. Biochem. Biophys., 259:52 (1987) and by Edge et al., Anal. Biochem., 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., Meth. Enzymol., 138:350 (1987).

Another type of covalent modification of PRO comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The PRO of the present invention may also be modified in a way to form a chimeric molecule comprising PRO fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO. The presence of such epitope-tagged forms of the PRO can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an  $\alpha$ -tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a PRO polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

#### D. Preparation of PRO

The description below relates primarily to production of PRO by culturing cells transformed or transfected with a vector containing PRO nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO. For instance, the PRO sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO.

##### 1. Isolation of DNA Encoding PRO

DNA encoding PRO may be obtained from a cDNA library prepared from tissue believed to possess the PRO mRNA and to express it at a detectable level. Accordingly, human PRO DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated nucleic acid synthesis).

Libraries can be screened with probes (such as antibodies to the PRO or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding PRO is to use PCR methodology [Sambrook et al., supra; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like <sup>32</sup>P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook et al., supra.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

## 2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., supra.

Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the ordinarily skilled artisan, for example,  $\text{CaCl}_2$ ,  $\text{CaPO}_4$ , liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan<sup>r</sup>*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7*

*ilvG kar'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning  
 5 or expression hosts for PRO-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, Nature, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer et al., Bio/Technology, 9:968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt et al., J. Bacteriol., 737 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC  
 10 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906; Van den Berg et al., Bio/Technology, 8:135 (1990)), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna et al., J. Basic Microbiol., 28:265-278 [1988]); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case et al., Proc. Natl. Acad. Sci. USA, 76:5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g.,  
 15 *Neurospora*, *Penicillium*, *Totipocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance et al., Biochem. Biophys. Res. Commun., 112:284-289 [1983]; Tilburn et al., Gene, 26:205-221 [1983]; Yelton et al., Proc. Natl. Acad. Sci. USA, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, EMBO J., 4:475-479 [1985]). Methylophilic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*,  
 20 *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, The Biochemistry of Methylophilic, 269 (1982).

Suitable host cells for the expression of glycosylated PRO are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells.  
 25 More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen. Virol., 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT  
 30 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

### 3. Selection and Use of a Replicable Vector

The nucleic acid (e.g., cDNA or genomic DNA) encoding PRO may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector  
 35 may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally

include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

5 The PRO may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g.,  
10 the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral  
15 secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the  $2\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for  
20 cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

25 An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb et al., Nature,  
30 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, Genetics, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well  
35 known. Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems [Chang et al., Nature, 275:615 (1978); Goeddel et al., Nature, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776], and hybrid

promoters such as the tac promoter [deBoer et al., Proc. Natl. Acad. Sci. USA, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO.

5 Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., J. Biol. Chem., 255:2073 (1980)] or other glycolytic enzymes [Hess et al., J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

10 Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

15 PRO transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

20 Transcription of a DNA encoding the PRO by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO coding sequence, but is preferably located at a site 5' from the promoter.

25 Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO.

30 Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO in recombinant vertebrate cell culture are described in Gething et al., Nature, 293:620-625 (1981); Mantei et al., Nature, 281:40-46 (1979); EP 117,060; and EP 117,058.

#### 4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO DNA and encoding a specific antibody epitope.

#### 5. Purification of Polypeptide

Forms of PRO may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or by enzymatic cleavage. Cells employed in expression of PRO can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and Practice, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO produced.

#### E. Uses for PRO

Nucleotide sequences (or their complement) encoding PRO have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. PRO nucleic acid will also be useful for the preparation of PRO polypeptides by the recombinant techniques described herein.

The full-length native sequence PRO gene, or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length PRO cDNA or to isolate still other cDNAs (for instance, those encoding naturally-occurring variants of PRO or PRO from other species) which have a desired sequence identity to the native PRO sequence disclosed herein. Optionally, the length of the probes will be about 20 to about 50 bases. The hybridization probes may be derived from at least partially novel regions of the full length native nucleotide sequence wherein those regions may be determined without undue experimentation or from genomic sequences including promoters, enhancer elements and introns of native sequence PRO. By way of example, a screening method will comprise isolating the coding region of the PRO gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the PRO gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine which members of such libraries the probe hybridizes to. Hybridization techniques are described in further detail in the Examples below.

Any EST sequences disclosed in the present application may similarly be employed as probes, using the methods disclosed herein.

Other useful fragments of the PRO nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target PRO mRNA (sense) or PRO DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of PRO DNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (Cancer Res. 48:2659, 1988) and van der Krol et al. (BioTechniques 6:958, 1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block transcription or translation of the target sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of PRO proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10048, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.



Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example,  $\text{CaPO}_4$ -mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. In a preferred procedure, an antisense or sense oligonucleotide is inserted into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either *in vivo* or *ex vivo*. Suitable retroviral  
5 vectors include, but are not limited to, those derived from the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see WO 90/13641).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable  
10 ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target  
15 nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related PRO coding sequences.

Nucleotide sequences encoding a PRO can also be used to construct hybridization probes for mapping  
20 the gene which encodes that PRO and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as *in situ* hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries.

When the coding sequences for PRO encode a protein which binds to another protein (example, where  
25 the PRO is a receptor), the PRO can be used in assays to identify the other proteins or molecules involved in the binding interaction. By such methods, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the receptor PRO can be used to isolate correlative ligand(s). Screening assays can be designed to find lead compounds that mimic the biological activity of a native PRO or  
30 a receptor for PRO. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

35 Nucleic acids which encode PRO or its modified forms can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (e.g., a mouse or rat) is an animal having cells that contain a transgene,

which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding PRO can be used to clone genomic DNA encoding PRO in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding PRO. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for PRO transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding PRO introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding PRO. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of PRO can be used to construct a PRO "knock out" animal which has a defective or altered gene encoding PRO as a result of homologous recombination between the endogenous gene encoding PRO and altered genomic DNA encoding PRO introduced into an embryonic stem cell of the animal. For example, cDNA encoding PRO can be used to clone genomic DNA encoding PRO in accordance with established techniques. A portion of the genomic DNA encoding PRO can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., *Cell*, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the PRO polypeptide.

Nucleic acid encoding the PRO polypeptides may also be used in gene therapy. In gene therapy applications, genes are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA.

Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. (Zamecnik *et al.*, Proc. Natl. Acad. Sci. USA 83:4143-4146 [1986]). The oligonucleotides can be modified to enhance their uptake, e.g. by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau *et al.*, Trends in Biotechnology 11, 205-210 [1993]). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g. capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, J. Biol. Chem. 262, 4429-4432 (1987); and Wagner *et al.*, Proc. Natl. Acad. Sci. USA 87, 3410-3414 (1990). For review of gene marking and gene therapy protocols see Anderson *et al.*, Science 256, 808-813 (1992).

The PRO polypeptides described herein may also be employed as molecular weight markers for protein electrophoresis purposes.

The nucleic acid molecules encoding the PRO polypeptides or fragments thereof described herein are useful for chromosome identification. In this regard, there exists an ongoing need to identify new chromosome markers, since relatively few chromosome marking reagents, based upon actual sequence data are presently available. Each PRO nucleic acid molecule of the present invention can be used as a chromosome marker.

The PRO polypeptides and nucleic acid molecules of the present invention may also be used for tissue typing, wherein the PRO polypeptides of the present invention may be differentially expressed in one tissue as compared to another. PRO nucleic acid molecules will find use for generating probes for PCR, Northern analysis, Southern analysis and Western analysis.

The PRO polypeptides described herein may also be employed as therapeutic agents. The PRO polypeptides of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the PRO product hereof is combined in admixture with a pharmaceutically acceptable carrier vehicle. Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the

dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, PLURONICS™ or PEG.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution.

Therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of administration is in accord with known methods, e.g. injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical administration, or by sustained release systems.

Dosages and desired drug concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" In *Toxicokinetics and New Drug Development*, Yacobi et al., Eds., Pergamon Press, New York 1989, pp. 42-96.

When *in vivo* administration of a PRO polypeptide or agonist or antagonist thereof is employed, normal dosage amounts may vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 µg/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

Where sustained-release administration of a PRO polypeptide is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of the PRO polypeptide, microencapsulation of the PRO polypeptide is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon- (rhIFN- ), interleukin-2, and MN rgp120. Johnson et al., Nat. Med., 2:795-799 (1996); Yasuda, Biomed. Ther., 27:1221-1223 (1993); Hora et al., Bio/Technology, 8:755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in Vaccine Design: The Subunit and Adjuvant Approach, Powell and Newman, eds, (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399; and U.S. Pat. No. 5,654,010.

The sustained-release formulations of these proteins were developed using poly-lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer," in: M. Chasin and  
5 R. Langer (Eds.), Biodegradable Polymers as Drug Delivery Systems (Marcel Dekker: New York, 1990), pp. 1-41.

This invention encompasses methods of screening compounds to identify those that mimic the PRO polypeptide (agonists) or prevent the effect of the PRO polypeptide (antagonists). Screening assays for antagonist drug candidates are designed to identify compounds that bind or complex with the PRO polypeptides  
10 encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well characterized in the art.

15 All assays for antagonists are common in that they call for contacting the drug candidate with a PRO polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the PRO polypeptide encoded by the gene identified herein or the  
20 drug candidate is immobilized on a solid phase, e.g., on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the PRO polypeptide and drying. Alternatively, an immobilized antibody, e.g., a monoclonal antibody, specific for the PRO polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized  
25 component, e.g., the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g., by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody  
30 specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular PRO polypeptide encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, e.g., cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers  
35 (Fields and Song, Nature (London), 340:245-246 (1989); Chien et al., Proc. Natl. Acad. Sci. USA, 88:9578-9582 (1991)) as disclosed by Chevray and Nathans, Proc. Natl. Acad. Sci. USA, 89: 5789-5793 (1991). Many

transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, the other one functioning as the transcription-activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for  $\beta$ -galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a gene encoding a PRO polypeptide identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a candidate compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

To assay for antagonists, the PRO polypeptide may be added to a cell along with the compound to be screened for a particular activity and the ability of the compound to inhibit the activity of interest in the presence of the PRO polypeptide indicates that the compound is an antagonist to the PRO polypeptide. Alternatively, antagonists may be detected by combining the PRO polypeptide and a potential antagonist with membrane-bound PRO polypeptide receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. The PRO polypeptide can be labeled, such as by radioactivity, such that the number of PRO polypeptide molecules bound to the receptor can be used to determine the effectiveness of the potential antagonist. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Coligan et al., Current Protocols in Immun., 1(2): Chapter 5 (1991). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the PRO polypeptide and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the PRO polypeptide. Transfected cells that are grown on glass slides are exposed to labeled PRO polypeptide. The PRO polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a

single clone that encodes the putative receptor.

As an alternative approach for receptor identification, labeled PRO polypeptide can be photoaffinity-linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the receptor can be excised, resolved into peptide fragments, and subjected to protein micro-sequencing. The amino acid sequence obtained  
5 from micro-sequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

In another assay for antagonists, mammalian cells or a membrane preparation expressing the receptor would be incubated with labeled PRO polypeptide in the presence of the candidate compound. The ability of the compound to enhance or block this interaction could then be measured.

10 More specific examples of potential antagonists include an oligonucleotide that binds to the fusions of immunoglobulin with PRO polypeptide, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the PRO  
15 polypeptide that recognizes the receptor but imparts no effect, thereby competitively inhibiting the action of the PRO polypeptide.

Another potential PRO polypeptide antagonist is an antisense RNA or DNA construct prepared using antisense technology, where, e.g., an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense technology can be used  
20 to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes the mature PRO polypeptides herein, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res.,  
25 6:3073 (1979); Cooney et al., Science, 241: 456 (1988); Dervan et al., Science, 251:1360 (1991)), thereby preventing transcription and the production of the PRO polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the PRO polypeptide (antisense - Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression (CRC Press: Boca Raton, FL, 1988). The oligonucleotides described above can also be delivered  
30 to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the PRO polypeptide. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation-initiationsite, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the PRO polypeptide, thereby blocking the normal biological  
35 activity of the PRO polypeptide. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, e.g., Rossi, Current Biology, 4:469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

5 Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g., PCT publication No. WO 97/33551, *supra*.

10 These small molecules can be identified by any one or more of the screening assays discussed hereinabove and/or by any other screening techniques well known for those skilled in the art.

PRO189 can be used in assays with W01A6.1 of *C. Elegans*, phosphodiesterases, transporters and proteins which bind to fatty acids, to determine the relative activities of PRO189 against these proteins. The results can be applied accordingly.

15

#### F. Anti-PRO Antibodies

The present invention further provides anti-PRO antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

20

##### 1. Polyclonal Antibodies

The anti-PRO antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

30 The immunization protocol may be selected by one skilled in the art without undue experimentation.

##### 2. Monoclonal Antibodies

The anti-PRO antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.



The immunizing agent will typically include the PRO polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103].

5 Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will  
10 include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk  
15 Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of  
20 monoclonal antibodies directed against PRO. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

25 After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture  
30 medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding  
35 specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells,

or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

*In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

### 3. Human and Humanized Antibodies

The anti-PRO antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers

[Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., Bio/Technology 10, 779-783 (1992); Lonberg et al., Nature 368 856-859 (1994); Morrison, Nature 368, 812-13 (1994); Fishwild et al., Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995).

#### 4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, Nature, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in

at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody  
5 molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large  
10 amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')<sub>2</sub> bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical  
15 linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an  
20 equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected  
25 to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various technique for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers.  
30 Kostelny et al., J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative  
35 mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with

the complementary  $V_L$  and  $V_H$  domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, J. Immunol. 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, J. Immunol. 147:60 (1991).

5 Exemplary bispecific antibodies may bind to two different epitopes on a given PRO polypeptide herein. Alternatively, an anti-PRO polypeptide arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular PRO polypeptide. Bispecific antibodies may also be used to localize  
10 cytotoxic agents to cells which express a particular PRO polypeptide. These antibodies possess a PRO-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the PRO polypeptide and further binds tissue factor (TF).

#### 15 5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro*  
20 using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

#### 25 6. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased  
30 complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, Anti-Cancer Drug Design, 3: 219-230 (1989).  
35

7. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above.

- 5 Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are  
10 available for the production of radioconjugated antibodies. Examples include  $^{212}\text{Bi}$ ,  $^{131}\text{I}$ ,  $^{131}\text{In}$ ,  $^{90}\text{Y}$ , and  $^{186}\text{Re}$ .

- Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-  
15 diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

- 20 In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is conjugated to a cytotoxic agent (e.g., a radionucleotide).

25 8. Immunoliposomes

- The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No.  
30 5,013,556.

- Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin  
35 *et al.*, J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, J. National Cancer Inst., 81(19): 1484 (1989).

## 9. Pharmaceutical Compositions of Antibodies

Antibodies specifically binding a PRO polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders in the form of pharmaceutical compositions.

If the PRO polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, *e.g.*, Marasco *et al.*, Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993). The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, *supra*.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

### G. Uses for anti-PRO Antibodies

The anti-PRO antibodies of the invention have various utilities. For example, anti-PRO antibodies may be used in diagnostic assays for PRO, *e.g.*, detecting its expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases

5 [Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , or  $^{125}\text{I}$ , a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or

10 horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter et al., Nature, 144:945 (1962); David et al., Biochemistry, 13:1014 (1974); Pain et al., J. Immunol. Meth., 40:219 (1981); and Nygren, J. Histochem. and Cytochem., 30:407 (1982).

Anti-PRO antibodies also are useful for the affinity purification of PRO from recombinant cell culture

15 or natural sources. In this process, the antibodies against PRO are immobilized on a suitable support, such as Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the PRO to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the PRO, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the PRO from the

20 antibody.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

### EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas,

30 VA.

#### EXAMPLE 1: Extracellular Domain Homology Screening to Identify Novel Polypeptides and cDNA Encoding Therefor

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about

35 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public databases (*e.g.*, Dayhoff, GenBank), and proprietary databases (*e.g.* LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program WU-BLAST-2



(Altschul et al., Methods in Enzymology 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons with a Blast score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, WA).

5 Using this extracellular domain homology screen, consensus DNA sequences were assembled relative to the other identified EST sequences using phrap. In addition, the consensus DNA sequences obtained were often (but not always) extended using repeated cycles of WU-BLAST-2 and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above.

Based upon the consensus sequences obtained as described above, oligonucleotides were then synthesized and used to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for a PRO polypeptide. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., Current Protocols in Molecular Biology, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

15 The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

#### EXAMPLE 2: Isolation of cDNA clones by Amylase Screening

##### 25 1. Preparation of oligo dT primed cDNA library

mRNA was isolated from a human tissue of interest using reagents and protocols from Invitrogen, San Diego, CA (Fast Track 2). This RNA was used to generate an oligo dT primed cDNA library in the vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). In this procedure, the double stranded cDNA was sized to greater than 1000 bp and the SalI/NotI linked cDNA was cloned into XhoI/NotI cleaved vector. pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites.

##### 2. Preparation of random primed cDNA library

35 A secondary cDNA library was generated in order to preferentially represent the 5' ends of the primary cDNA clones. Sp6 RNA was generated from the primary library (described above), and this RNA was used to generate a random primed cDNA library in the vector pSST-AMY.0 using reagents and protocols from Life Technologies (Super Script Plasmid System, referenced above). In this procedure the double stranded cDNA

was sized to 500-1000 bp, linked with blunt to NotI adaptors, cleaved with SfiI, and cloned into SfiI/NotI cleaved vector. pSST-AMY.0 is a cloning vector that has a yeast alcohol dehydrogenase promoter preceding the cDNA cloning sites and the mouse amylase sequence (the mature sequence without the secretion signal) followed by the yeast alcohol dehydrogenase terminator, after the cloning sites. Thus, cDNAs cloned into this vector that are fused in frame with amylase sequence will lead to the secretion of amylase from appropriately transfected yeast colonies.

### 3. Transformation and Detection

DNA from the library described in paragraph 2 above was chilled on ice to which was added electrocompetent DH10B bacteria (Life Technologies, 20 ml). The bacteria and vector mixture was then electroporated as recommended by the manufacturer. Subsequently, SOC media (Life Technologies, 1 ml) was added and the mixture was incubated at 37°C for 30 minutes. The transformants were then plated onto 20 standard 150 mm LB plates containing ampicillin and incubated for 16 hours (37°C). Positive colonies were scraped off the plates and the DNA was isolated from the bacterial pellet using standard protocols, e.g. CsCl-gradient. The purified DNA was then carried on to the yeast protocols below.

The yeast methods were divided into three categories: (1) Transformation of yeast with the plasmid/cDNA combined vector; (2) Detection and isolation of yeast clones secreting amylase; and (3) PCR amplification of the insert directly from the yeast colony and purification of the DNA for sequencing and further analysis.

The yeast strain used was HD56-5A (ATCC-90785). This strain has the following genotype: MAT alpha, ura3-52, leu2-3, leu2-112, his3-11, his3-15, MAL<sup>+</sup>, SUC<sup>+</sup>, GAL<sup>+</sup>. Preferably, yeast mutants can be employed that have deficient post-translational pathways. Such mutants may have translocation deficient alleles in *sec71*, *sec72*, *sec62*, with truncated *sec71* being most preferred. Alternatively, antagonists (including antisense nucleotides and/or ligands) which interfere with the normal operation of these genes, other proteins implicated in this post translation pathway (e.g., SEC61p, SEC72p, SEC62p, SEC63p, TDJ1p or SSA1p-4p) or the complex formation of these proteins may also be preferably employed in combination with the amylase-expressing yeast.

Transformation was performed based on the protocol outlined by Gietz et al., Nucl. Acid. Res., 20:1425 (1992). Transformed cells were then inoculated from agar into YEPD complex media broth (100 ml) and grown overnight at 30°C. The YEPD broth was prepared as described in Kaiser et al., Methods in Yeast Genetics, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 207 (1994). The overnight culture was then diluted to about  $2 \times 10^6$  cells/ml (approx. OD<sub>600</sub>=0.1) into fresh YEPD broth (500 ml) and regrown to  $1 \times 10^7$  cells/ml (approx. OD<sub>600</sub>=0.4-0.5).

The cells were then harvested and prepared for transformation by transfer into GS3 rotor bottles in a Sorval GS3 rotor at 5,000 rpm for 5 minutes, the supernatant discarded, and then resuspended into sterile water, and centrifuged again in 50 ml falcon tubes at 3,500 rpm in a Beckman GS-6KR centrifuge. The supernatant was discarded and the cells were subsequently washed with LiAc/TE (10 ml, 10 mM Tris-HCl, 1 mM EDTA pH 7.5, 100 mM Li<sub>2</sub>OOCCH<sub>3</sub>), and resuspended into LiAc/TE (2.5 ml).

Transformation took place by mixing the prepared cells (100  $\mu$ l) with freshly denatured single stranded salmon testes DNA (Lofstrand Labs, Gaithersburg, MD) and transforming DNA (1  $\mu$ g, vol. < 10  $\mu$ l) in microfuge tubes. The mixture was mixed briefly by vortexing, then 40% PEG/TE (600  $\mu$ l, 40% polyethylene glycol-4000, 10 mM Tris-HCl, 1 mM EDTA, 100 mM Li<sub>2</sub>OOCCH<sub>3</sub>, pH 7.5) was added. This mixture was gently mixed and incubated at 30°C while agitating for 30 minutes. The cells were then heat shocked at 42°C for 15 minutes, and the reaction vessel centrifuged in a microfuge at 12,000 rpm for 5-10 seconds, decanted and resuspended into TE (500  $\mu$ l, 10 mM Tris-HCl, 1 mM EDTA pH 7.5) followed by recentrifugation. The cells were then diluted into TE (1 ml) and aliquots (200  $\mu$ l) were spread onto the selective media previously prepared in 150 mm growth plates (VWR).

Alternatively, instead of multiple small reactions, the transformation was performed using a single, large scale reaction, wherein reagent amounts were scaled up accordingly.

The selective media used was a synthetic complete dextrose agar lacking uracil (SCD-Ura) prepared as described in Kaiser et al., Methods in Yeast Genetics, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 208-210 (1994). Transformants were grown at 30°C for 2-3 days.

The detection of colonies secreting amylase was performed by including red starch in the selective growth media. Starch was coupled to the red dye (Reactive Red-120, Sigma) as per the procedure described by Biely et al., Anal. Biochem., 172:176-179 (1988). The coupled starch was incorporated into the SCD-Ura agar plates at a final concentration of 0.15% (w/v), and was buffered with potassium phosphate to a pH of 7.0 (50-100 mM final concentration).

The positive colonies were picked and streaked across fresh selective media (onto 150 mm plates) in order to obtain well isolated and identifiable single colonies. Well isolated single colonies positive for amylase secretion were detected by direct incorporation of red starch into buffered SCD-Ura agar. Positive colonies were determined by their ability to break down starch resulting in a clear halo around the positive colony visualized directly.

#### 4. Isolation of DNA by PCR Amplification

When a positive colony was isolated, a portion of it was picked by a toothpick and diluted into sterile water (30  $\mu$ l) in a 96 well plate. At this time, the positive colonies were either frozen and stored for subsequent analysis or immediately amplified. An aliquot of cells (5  $\mu$ l) was used as a template for the PCR reaction in a 25  $\mu$ l volume containing: 0.5  $\mu$ l Klentaq (Clontech, Palo Alto, CA); 4.0  $\mu$ l 10 mM dNTP's (Perkin Elmer-Cetus); 2.5  $\mu$ l Kentaq buffer (Clontech); 0.25  $\mu$ l forward oligo 1; 0.25  $\mu$ l reverse oligo 2; 12.5  $\mu$ l distilled water. The sequence of the forward oligonucleotide 1 was:

5'-TGTAACGACGGCCAGTTAAATAGACCTGCAATTATTAATCT-3' (SEQ ID NO:3)

The sequence of reverse oligonucleotide 2 was:

5'-CAGGAAACAGCTATGACCACCTGCACACCTGCAAATCCATT-3' (SEQ ID NO:4)

PCR was then performed as follows:

- a. Denature 92°C, 5 minutes
- b. 3 cycles of: Denature 92°C, 30 seconds

- |    |    |               |       |            |
|----|----|---------------|-------|------------|
|    |    | Anneal        | 59°C, | 30 seconds |
|    |    | Extend        | 72°C, | 60 seconds |
| 5  | c. | 3 cycles of:  |       |            |
|    |    | Denature      | 92°C, | 30 seconds |
|    |    | Anneal        | 57°C, | 30 seconds |
|    |    | Extend        | 72°C, | 60 seconds |
| 10 | d. | 25 cycles of: |       |            |
|    |    | Denature      | 92°C, | 30 seconds |
|    |    | Anneal        | 55°C, | 30 seconds |
|    |    | Extend        | 72°C, | 60 seconds |
|    | e. | Hold          | 4°C   |            |

The underlined regions of the oligonucleotides annealed to the ADH promoter region and the amylase region, respectively, and amplified a 307 bp region from vector pSST-AMY.0 when no insert was present. Typically, the first 18 nucleotides of the 5' end of these oligonucleotides contained annealing sites for the sequencing primers. Thus, the total product of the PCR reaction from an empty vector was 343 bp. However, signal sequence-fused cDNA resulted in considerably longer nucleotide sequences.

Following the PCR, an aliquot of the reaction (5 µl) was examined by agarose gel electrophoresis in a 1% agarose gel using a Tris-Borate-EDTA (TBE) buffering system as described by Sambrook et al., *supra*. Clones resulting in a single strong PCR product larger than 400 bp were further analyzed by DNA sequencing after purification with a 96 Qiaquick PCR clean-up column (Qiagen Inc., Chatsworth, CA).

#### EXAMPLE 3: Isolation of cDNA Clones Using Signal Algorithm Analysis

Various polypeptide-encoding nucleic acid sequences were identified by applying a proprietary signal sequence finding algorithm developed by Genentech, Inc. (South San Francisco, CA) upon ESTs as well as clustered and assembled EST fragments from public (e.g., GenBank) and/or private (LIFESEQ®, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion signals. Use of this algorithm resulted in the identification of numerous polypeptide-encoding nucleic acid sequences.

#### EXAMPLE 4: Isolation of cDNA clones Encoding Human PRO281

In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., *Proc. Natl. Acad. Sci. USA* 93:7108-7113 (1996) were employed with the following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of *E. coli* as

MUPM\_MOUSE, MUP\_RAT and ECU70823\_1.

**EXAMPLE 76: Isolation of cDNA clones Encoding Human PRO994**

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database, designated 157555. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., *Methods in Enzymology* 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA55728.

In light of an observed sequence homology between the DNA55728 consensus sequence and an EST sequence encompassed within the Incyte EST clone no. 2860366, the Incyte EST clone 2860366 was purchased and the cDNA insert was obtained and sequenced. It was found that this insert encoded a full-length protein. The sequence of this cDNA insert is shown in Figure 175 and is herein designated as DNA58855-1422.

Clone DNA58855-1422 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 31-33 and ending at the stop codon at nucleotide positions 718-720 (Figure 175). The predicted polypeptide precursor is 229 amino acids long (Figure 176). The full-length PRO994 protein shown in Figure 176 has an estimated molecular weight of about 25,109 daltons and a pI of about 6.83. Analysis of the full-length PRO994 sequence shown in Figure 176 (SEQ ID NO:258) evidences the presence of the following: transmembrane domains from about amino acid 10 to about amino acid 31, from about amino acid 50 to about amino acid 72, from about amino acid 87 to about amino acid 110 and from about amino acid 191 to about amino acid 213, potential N-glycosylation sites from about amino acid 80 to about amino acid 83, from about amino acid 132 to about amino acid 135, from about amino acid 148 to about amino acid 151 and from about amino acid 163 to about amino acid 166 and an amino acid block having homology to TNFR/NGFR cysteine-rich region proteins from about amino acid 4 to about amino acid 11. Clone DNA58855-1422 has been deposited with ATCC on June 23, 1998 and is assigned ATCC deposit no. 203018.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 176 (SEQ ID NO:258), evidenced significant homology between the PRO994 amino acid sequence and the following Dayhoff sequences: AF027204\_1, TAL6\_HUMAN, ILT4\_HUMAN, JC6205, MMU57570\_1, S40363, ETU56093\_1, S42858, P\_R66849 and P\_R74751.

**EXAMPLE 77: Isolation of cDNA clones Encoding Human PRO812**

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database, designated 170079. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank)

and a proprietary EST DNA database (Lifeseq®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated as DNA55721.

In light of an observed sequence homology between the DNA55721 consensus sequence and an EST sequence encompassed within the Incyte EST clone no. 388964, the Incyte EST clone 388964 was purchased and the cDNA insert was obtained and sequenced. It was found that this insert encoded a full-length protein. The sequence of this cDNA insert is shown in Figure 177 and is herein designated as DNA59205-1421.

Clone DNA59205-1421 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 55-57 and ending at the stop codon at nucleotide positions 304-306 (Figure 177). The predicted polypeptide precursor is 83 amino acids long (Figure 178). The full-length PRO812 protein shown in Figure 178 has an estimated molecular weight of about 9,201 daltons and a pI of about 9.3. Analysis of the full-length PRO812 sequence shown in Figure 178 (SEQ ID NO:260) evidences the presence of the following:

a signal peptide from about amino acid 1 to about amino acid 15, a cAMP- and cGMP-dependent protein kinase phosphorylation site from about amino acid 73 to about amino acid 76 and protein kinase C phosphorylation sites from about amino acid 70 to about amino acid 72 and from about amino acid 76 to about amino acid 78. Clone DNA59205-1421 has been deposited with ATCC on June 23, 1998 and is assigned ATCC deposit no. 203009.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 178 (SEQ ID NO:260), evidenced significant homology between the PRO812 amino acid sequence and the following Dayhoff sequences: P\_W35802, P\_W35803, PSC1\_RAT, S68231, GEN13917, PSC2\_RAT, CC10\_HUMAN, UTER\_RABBIT, AF008595\_1 and A56413.

#### EXAMPLE 78: Isolation of cDNA clones Encoding Human PRO1069

Use of the signal sequence algorithm described in Example 3 above allowed identification of a single Incyte EST sequence designated herein as 100727. This sequence was then compared to a proprietary EST DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, Univ. of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA56001.

In light of an observed sequence homology between the DNA56001 consensus sequence and an EST sequence encompassed within the Incyte EST clone no. 3533881, the Incyte EST clone 3533881 was purchased and the cDNA insert was obtained and sequenced. It was found that this insert encoded a full-length protein. The sequence of this cDNA insert is shown in Figure 179 and is the full-length DNA sequence for PRO1069.

and NK13\_RAT.

Clone DNA71159-1617 has been deposited with ATCC and is assigned ATCC deposit no. 203135.

**EXAMPLE 139: Use of PRO as a hybridization probe**

The following method describes use of a nucleotide sequence encoding PRO as a hybridization probe.

5 DNA comprising the coding sequence of full-length or mature PRO as disclosed herein is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO can then be identified using standard techniques known in the art.

15

**EXAMPLE 140: Expression of PRO in *E. coli***

This example illustrates preparation of an unglycosylated form of PRO by recombinant expression in *E. coli*.

The DNA sequence encoding PRO is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar et al., *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook et al., *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

35 After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO protein can then be purified using a metal chelating column under conditions that allow tight binding of the

protein.

PRO may be expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding PRO is initially amplified using selected PCR primers. The primers will contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences are then ligated into an expression vector, which is used to transform an *E. coli* host based on strain 52 (W3110 *fuH*A(*ton*A) *lon* *gal*E *rpo*Hts(*htp*Rts) *clp*P(*lac*Iq)). Transformants are first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 is reached. Cultures are then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.71 g sodium citrate•2H<sub>2</sub>O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM MgSO<sub>4</sub>) and grown for approximately 20-30 hours at 30°C with shaking. Samples are removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets are frozen until purification and refolding.

*E. coli* paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentrifuge for 30 min. The supernatant is diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. The clarified extract is loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein is eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein are pooled and stored at 4°C. Protein concentration is estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are refolded by diluting the sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes are chosen so that the final protein concentration is between 50 to 100 micrograms/ml. The refolding solution is stirred gently at 4°C for 12-36 hours. The refolding reaction is quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution is filtered through a 0.22 micron filter and acetonitrile is added to 2-10% final concentration. The refolded protein is chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance are analyzed on SDS polyacrylamide gels and fractions containing homogeneous refolded protein are pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin



from the samples.

Fractions containing the desired folded PRO polypeptide are pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins are formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

- 5 Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

**EXAMPLE 141: Expression of PRO in mammalian cells**

This example illustrates preparation of a potentially glycosylated form of PRO by recombinant expression in mammalian cells.

- 10 The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO DNA using ligation methods such as described in Sambrook et al., *supra*. The resulting vector is called pRK5-PRO.

- 15 In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10  $\mu$ g pRK5-PRO DNA is mixed with about 1  $\mu$ g DNA encoding the VA RNA gene [Thimmappaya et al., *Cell*, 31:543 (1982)] and dissolved in 500  $\mu$ l of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M  $\text{CaCl}_2$ . To this mixture is added, dropwise, 500  $\mu$ l of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM  $\text{NaPO}_4$ , and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

- 25 Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200  $\mu$ Ci/ml  $^{35}\text{S}$ -cysteine and 200  $\mu$ Ci/ml  $^{35}\text{S}$ -methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

- 30 In an alternative technique, PRO may be introduced into 293 cells transiently using the dextran sulfate method described by Somparyrac et al., *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700  $\mu$ g pRK5-PRO DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5  $\mu$ g/ml bovine insulin and 35 0.1  $\mu$ g/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO can be expressed in CHO cells. The pRK5-PRO can be transfected into CHO cells using known reagents such as  $\text{CaPO}_4$  or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as  $^{35}\text{S}$ -methionine. After determining the presence of PRO polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO can then be concentrated and purified by any selected method.

Epitope-tagged PRO may also be expressed in host CHO cells. The PRO may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO can then be concentrated and purified by any selected method, such as by  $\text{Ni}^{2+}$ -chelate affinity chromatography.

PRO may also be expressed in CHO and/or COS cells by a transient expression procedure or in CHO cells by another stable expression procedure.

Stable expression in CHO cells is performed using the following procedure. The proteins are expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. extracellular domains) of the respective proteins are fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

Following PCR amplification, the respective DNAs are subcloned in a CHO expression vector using standard techniques as described in Ausubel et al., Current Protocols of Molecular Biology, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas et al., Nucl. Acids Res. 24:9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect\* (Quiagen), Dosper\* or Fugene\* (Boehringer Mannheim). The cells are grown as described in Lucas et al., supra. Approximately  $3 \times 10^7$  cells are frozen in an ampule for further growth and production as described below.

The ampules containing the plasmid DNA are thawed by placement into water bath and mixed by vortexing. The contents are pipetted into a centrifuge tube containing 10 mLs of media and centrifuged at 1000 rpm for 5 minutes. The supernatant is aspirated and the cells are resuspended in 10 mL of selective media (0.2  $\mu\text{m}$  filtered PS20 with 5% 0.2  $\mu\text{m}$  diafiltered fetal bovine serum). The cells are then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells are transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, 250 mL, 500 mL and 2000 mL spinners are seeded with  $3 \times 10^5$  cells/mL. The cell media is exchanged with fresh media by

centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in U.S. Patent No. 5,122,469, issued June 16, 1992 may actually be used. A 3L production spinner is seeded at  $1.2 \times 10^6$  cells/mL. On day 0, the cell number pH is determined. On day 1, the spinner is sampled and sparging with filtered air is commenced. On day 2, the spinner is sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (e.g., 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion) taken. Throughout the production, the pH is adjusted as necessary to keep it at around 7.2. After 10 days, or until the viability dropped below 70%, the cell culture is harvested by centrifugation and filtering through a 0.22  $\mu$ m filter. The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins are purified using a Ni-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc-containing) constructs are purified from the conditioned media as follows. The conditioned medium is pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275  $\mu$ L of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

#### EXAMPLE 142: Expression of PRO in Yeast

The following method describes recombinant expression of PRO in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO from the ADH2/GAPDH promoter. DNA encoding PRO and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of PRO. For secretion, DNA encoding PRO can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO may further be purified using selected column chromatography resins.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

5 **EXAMPLE 143: Expression of PRO in Baculovirus-Infected Insect Cells**

The following method describes recombinant expression of PRO in Baculovirus-infected insect cells.

10 The sequence coding for PRO is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO or the desired portion of the coding sequence of PRO such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

15 Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994).

20 Expressed poly-his tagged PRO can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., Nature, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 μm filter. A Ni<sup>2+</sup>-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A<sub>280</sub> with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A<sub>280</sub> baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni<sup>2+</sup>-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His<sub>10</sub>-tagged PRO are pooled and dialyzed against loading buffer.

35 Alternatively, purification of the IgG tagged (or Fc tagged) PRO can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

**EXAMPLE 144: Preparation of Antibodies that Bind PRO**

This example illustrates preparation of monoclonal antibodies which can specifically bind PRO.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO, fusion proteins containing PRO, and cells expressing recombinant PRO on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

**EXAMPLE 145: Purification of PRO Polypeptides Using Specific Antibodies**

Native or recombinant PRO polypeptides may be purified by a variety of standard techniques in the art of protein purification. For example, pro-PRO polypeptide, mature PRO polypeptide, or pre-PRO polypeptide is purified by immunoaffinity chromatography using antibodies specific for the PRO polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-PRO polypeptide antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated SEPHAROSE™ (Pharmacia LKB Biotechnology). The antibody

is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of PRO polypeptide by preparing a fraction from cells containing PRO polypeptide in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble PRO polypeptide containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble PRO polypeptide-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRO polypeptide (*e.g.*, high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/PRO polypeptide binding (*e.g.*, a low pH buffer such as approximately pH 2-3, or a high concentration of a chaotrope such as urea or thiocyanate ion), and PRO polypeptide is collected.

#### EXAMPLE 146: Drug Screening

This invention is particularly useful for screening compounds by using PRO polypeptides or binding fragment thereof in any of a variety of drug screening techniques. The PRO polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the PRO polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between PRO polypeptide or a fragment and the agent being tested. Alternatively, one can examine the diminution in complex formation between the PRO polypeptide and its target cell or target receptors caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect a PRO polypeptide-associated disease or disorder. These methods comprise contacting such an agent with an PRO polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the PRO polypeptide or fragment, or (ii) for the presence of a complex between the PRO polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, the PRO polypeptide or fragment is typically labeled. After suitable incubation, free PRO polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to PRO polypeptide or to interfere with the PRO polypeptide/cell complex.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a PRO polypeptide, the peptide test compounds are reacted with PRO polypeptide and washed. Bound PRO polypeptide is detected by methods well known in the art. Purified PRO polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the

solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding PRO polypeptide specifically compete with a test compound for binding to PRO polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRO polypeptide.

5

EXAMPLE 147: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (*i.e.*, a PRO polypeptide) or of small molecules with which they interact, *e.g.*, agonists, antagonists, or inhibitors. Any of these examples can be used to fashion drugs which are more active or stable forms of the PRO polypeptide or which enhance or interfere with the function of the PRO polypeptide *in vivo* (*c.f.*, Hodgson, Bio/Technology, 9: 19-21 (1991)).

In one approach, the three-dimensional structure of the PRO polypeptide, or of an PRO polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the PRO polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of the PRO polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous PRO polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton and Wells, Biochemistry, 31:7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda *et al.*, J. Biochem., 113:742-746 (1993).

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

By virtue of the present invention, sufficient amounts of the PRO polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRO polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

35

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

Table 2

<u>5</u>	<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
	DNA16422-1209	209929	June 2, 1998
	DNA16435-1208	209930	June 2, 1998
	DNA21624-1391	209917	June 2, 1998
	DNA23334-1392	209918	June 2, 1998
10	DNA26288-1239	209792	April 21, 1998
	DNA26843-1389	203099	August 4, 1998
	DNA26844-1394	209926	June 2, 1998
	DNA30862-1396	209920	June 2, 1998
	DNA35680-1212	209790	April 21, 1998
15	DNA40621-1440	209922	June 2, 1998
	DNA44161-1434	209907	May 27, 1998
	DNA44694-1500	203114	August 11, 1998
	DNA45495-1550	203156	August 25, 1998
	DNA47361-1154	209431	November 7, 1997
20	DNA47394-1572	203109	August 11, 1998
	DNA48320-1433	209904	May 27, 1998
	DNA48334-1435	209924	June 2, 1998
	DNA48606-1479	203040	July 1, 1998
	DNA49141-1431	203003	June 23, 1998
25	DNA49142-1430	203002	June 23, 1998
	DNA49143-1429	203013	June 23, 1998
	DNA49647-1398	209919	June 2, 1998
	DNA49819-1439	209931	June 2, 1998
	DNA49820-1427	209932	June 2, 1998
30	DNA49821-1562	209981	June 16, 1998
	DNA52192-1369	203042	July 1, 1998
	DNA52598-1518	203107	August 11, 1998
	DNA53913-1490	203162	August 25, 1998
	DNA53978-1443	209983	June 16, 1998
35	DNA53996-1442	209921	June 2, 1998
	DNA56041-1416	203012	June 23, 1998
	DNA56047-1456	209948	June 9, 1998
	DNA56050-1455	203011	June 23, 1998
	DNA56110-1437	203113	August 11, 1998
40	DNA56113-1378	203049	July 1, 1998
	DNA56410-1414	209923	June 2, 1998
	DNA56436-1448	209902	May 27, 1998
	DNA56855-1447	203004	June 23, 1998
	DNA56859-1445	203019	June 23, 1998
45	DNA56860-1510	209952	June 9, 1998
	DNA56865-1491	203022	June 23, 1998
	DNA56866-1342	203023	June 23, 1998
	DNA56868-1209	203024	June 23, 1998
	DNA56869-1545	203161	August 25, 1998
50	DNA56870-1492	209925	June 2, 1998
	DNA57033-1403	209905	May 27, 1998
	DNA57037-1444	209903	May 27, 1998
	DNA57129-1413	209977	June 16, 1998



	DNA57690-1374	209950	June 9, 1998
	DNA57693-1424	203008	June 23, 1998
	DNA57694-1341	203017	June 23, 1998
	DNA57695-1340	203006	June 23, 1998
	DNA57699-1412	203020	June 23, 1998
5	DNA57702-1476	209951	June 9, 1998
	DNA57704-1452	209953	June 9, 1998
	DNA57708-1411	203021	June 23, 1998
	DNA57710-1451	203048	July 1, 1998
	DNA57711-1501	203047	July 1, 1998
10	DNA57827-1493	203045	July 1, 1998
	DNA57834-1339	209954	June 9, 1998
	DNA57836-1338	203025	June 23, 1998
	DNA57838-1337	203014	June 23, 1998
	DNA57844-1410	203010	June 23, 1998
15	DNA58721-1475	203110	August 11, 1998
	DNA58723-1588	203133	August 18, 1998
	DNA58737-1473	203136	August 18, 1998
	DNA58743-1609	203154	August 25, 1998
	DNA58846-1409	209957	June 9, 1998
20	DNA58848-1472	209955	June 9, 1998
	DNA58849-1494	209958	June 9, 1998
	DNA58850-1495	209956	June 9, 1998
	DNA58853-1423	203016	June 23, 1998
	DNA58855-1422	203018	June 23, 1998
25	DNA59205-1421	203009	June 23, 1998
	DNA59211-1450	209960	June 9, 1998
	DNA59213-1487	209959	June 9, 1998
	DNA59214-1449	203046	July 1, 1998
	DNA59215-1425	209961	June 9, 1998
30	DNA59220-1514	209962	June 9, 1998
	DNA59488-1603	203157	August 25, 1998
	DNA59493-1420	203050	July 1, 1998
	DNA59497-1496	209941	June 4, 1998
	DNA59588-1571	203106	August 11, 1998
35	DNA59603-1419	209944	June 9, 1998
	DNA59605-1418	203005	June 23, 1998
	DNA59606-1471	209945	June 9, 1998
	DNA59607-1497	209957	June 9, 1998
	DNA59609-1470	209963	June 9, 1998
40	DNA59610-1559	209990	June 16, 1998
	DNA59612-1466	209947	June 9, 1998
	DNA59613-1417	203007	June 23, 1998
	DNA59616-1465	209991	June 16, 1998
	DNA59619-1464	203041	July 1, 1998
45	DNA59620-1463	209989	June 16, 1998
	DNA59625-1498	209992	June 17, 1998
	DNA59767-1489	203108	August 11, 1998
	DNA59776-1600	203128	August 18, 1998
	DNA59777-1480	203111	August 11, 1998
50	DNA59820-1549	203129	August 18, 1998
	DNA59827-1426	203089	August 4, 1998
	DNA59828-1608	203158	August 25, 1998
	DNA59838-1462	209976	June 16, 1998
	DNA59839-1461	209988	June 16, 1998
55	DNA59841-1460	203044	July 1, 1998
	DNA59842-1502	209982	June 16, 1998

	DNA59846-1503	209978	June 16, 1998
	DNA59847-1511	203098	August 4, 1998
	DNA59848-1512	203088	August 4, 1998
	DNA59849-1504	209986	June 16, 1998
	DNA59853-1505	209985	June 16, 1998
5	DNA59854-1459	209974	June 16, 1998
	DNA60283-1484	203043	July 1, 1998
	DNA60615-1483	209980	June 16, 1998
	DNA60619-1482	209993	June 16, 1998
	DNA60621-1516	203091	August 4, 1998
10	DNA60622-1525	203090	August 4, 1998
	DNA60625-1507	209975	June 16, 1998
	DNA60627-1508	203092	August 4, 1998
	DNA60629-1481	209979	June 16, 1998
	DNA61755-1554	203112	August 11, 1998
15	DNA61873-1574	203132	August 18, 1998
	DNA62814-1521	203093	August 4, 1998
	DNA62872-1509	203100	August 4, 1998
	DNA62876-1517	203095	August 4, 1998
	DNA62881-1515	203096	August 4, 1998
20	DNA64852-1589	203127	August 18, 1998
	DNA64884-1527	203155	August 25, 1998
	DNA64890-1612	203131	August 18, 1998
	DNA65412-1523	203094	August 4, 1998
	DNA66308-1537	203159	August 25, 1998
25	DNA66309-1538	203235	September 15, 1998
	DNA67004-1614	203115	August 11, 1998
	DNA68869-1610	203164	August 25, 1998
	DNA68872-1620	203160	August 25, 1998
30	DNA71159-1617	203135	August 18, 1998

These deposit were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposits will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the

deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition  
5 to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. Isolated nucleic acid having at least 80% sequence identity to a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:6), Figure 6 (SEQ ID NO:8), Figure 9 (SEQ ID NO:14), Figure 12 (SEQ ID NO:20), Figure 15 (SEQ ID NO:23), Figure 18 (SEQ ID NO:28), Figure 20 (SEQ ID NO:30), Figure 23 (SEQ ID NO:33), Figure 25 (SEQ ID NO:36), Figure 27 (SEQ ID NO:41), Figure 30 (SEQ ID NO:47), Figure 32 (SEQ ID NO:52), Figure 34 (SEQ ID NO:57), Figure 36 (SEQ ID NO:62), Figure 38 (SEQ ID NO:67), Figure 41 (SEQ ID NO:73), Figure 47 (SEQ ID NO:84), Figure 49 (SEQ ID NO:95), Figure 51 (SEQ ID NO:97), Figure 53 (SEQ ID NO:99), Figure 57 (SEQ ID NO:103), Figure 64 (SEQ ID NO:113), Figure 66 (SEQ ID NO:115), Figure 68 (SEQ ID NO:117), Figure 70 (SEQ ID NO:119), Figure 72 (SEQ ID NO:124), Figure 74 (SEQ ID NO:129), Figure 76 (SEQ ID NO:135), Figure 79 (SEQ ID NO:138), Figure 83 (SEQ ID NO:146), Figure 85 (SEQ ID NO:148), Figure 88 (SEQ ID NO:151), Figure 90 (SEQ ID NO:153), Figure 93 (SEQ ID NO:156), Figure 95 (SEQ ID NO:158), Figure 97 (SEQ ID NO:160), Figure 99 (SEQ ID NO:165), Figure 101 (SEQ ID NO:167), Figure 103 (SEQ ID NO:169), Figure 105 (SEQ ID NO:171), Figure 109 (SEQ ID NO:175), Figure 111 (SEQ ID NO:177), Figure 113 (SEQ ID NO:179), Figure 115 (SEQ ID NO:181), Figure 117 (SEQ ID NO:183), Figure 120 (SEQ ID NO:189), Figure 122 (SEQ ID NO:194), Figure 125 (SEQ ID NO:197), Figure 127 (SEQ ID NO:199), Figure 129 (SEQ ID NO:201), Figure 131 (SEQ ID NO:203), Figure 133 (SEQ ID NO:205), Figure 135 (SEQ ID NO:207), Figure 137 (SEQ ID NO:209), Figure 139 (SEQ ID NO:211), Figure 141 (SEQ ID NO:213), Figure 144 (SEQ ID NO:216), Figure 147 (SEQ ID NO:219), Figure 149 (SEQ ID NO:221), Figure 151 (SEQ ID NO:223), Figure 153 (SEQ ID NO:225), Figure 155 (SEQ ID NO:227), Figure 157 (SEQ ID NO:229), Figure 159 (SEQ ID NO:231), Figure 161 (SEQ ID NO:236), Figure 163 (SEQ ID NO:241), Figure 165 (SEQ ID NO:246), Figure 167 (SEQ ID NO:248), Figure 169 (SEQ ID NO:250), Figure 171 (SEQ ID NO:253), Figure 174 (SEQ ID NO:256), Figure 176 (SEQ ID NO:258), Figure 178 (SEQ ID NO:260), Figure 180 (SEQ ID NO:262), Figure 182 (SEQ ID NO:264), Figure 184 (SEQ ID NO:266), Figure 186 (SEQ ID NO:268), Figure 188 (SEQ ID NO:270), Figure 190 (SEQ ID NO:272), Figure 192 (SEQ ID NO:274), Figure 194 (SEQ ID NO:276), Figure 196 (SEQ ID NO:278), Figure 198 (SEQ ID NO:281), Figure 200 (SEQ ID NO:283), Figure 202 (SEQ ID NO:285), Figure 204 (SEQ ID NO:287), Figure 206 (SEQ ID NO:289), Figure 208 (SEQ ID NO:291), Figure 210 (SEQ ID NO:293), Figure 212 (SEQ ID NO:295), Figure 214 (SEQ ID NO:297), Figure 216 (SEQ ID NO:299), Figure 218 (SEQ ID NO:301), Figure 220 (SEQ ID NO:303), Figure 226 (SEQ ID NO:309), Figure 228 (SEQ ID NO:314), Figure 230 (SEQ ID NO:319), Figure 233 (SEQ ID NO:326), Figure 235 (SEQ ID NO:334), Figure 238 (SEQ ID NO:340), Figure 240 (SEQ ID NO:345), Figure 242 (SEQ ID NO:347), Figure 244 (SEQ ID NO:349), Figure 246 (SEQ ID NO:351), Figure 248 (SEQ ID NO:353), Figure 250 (SEQ ID NO:355), Figure 252 (SEQ ID NO:357), Figure 254 (SEQ ID NO:359), Figure 256 (SEQ ID NO:361), Figure 258 (SEQ ID NO:363), Figure 260 (SEQ ID NO:365), Figure 262 (SEQ ID NO:367), Figure 264 (SEQ ID NO:369), Figure 266 (SEQ ID NO:371), Figure 268 (SEQ ID NO:373), Figure 270 (SEQ ID NO:375), Figure 272 (SEQ ID NO:377), Figure 274 (SEQ ID NO:379), Figure 276 (SEQ ID NO:381), Figure 278 (SEQ ID NO:387), Figure 280 (SEQ ID NO:389), Figure 282 (SEQ ID NO:394), Figure 284 (SEQ ID NO:399), Figure 286 (SEQ

ID NO:401), Figure 288 (SEQ ID NO:403), Figure 290 (SEQ ID NO:408), Figure 292 (SEQ ID NO:410), Figure 294 (SEQ ID NO:412), Figure 296 (SEQ ID NO:414), Figure 298 (SEQ ID NO:416), Figure 300 (SEQ ID NO:418), Figure 302 (SEQ ID NO:420), Figure 304 (SEQ ID NO:422) and Figure 306 (SEQ ID NO:424).

2. The nucleic acid sequence of Claim 1, wherein said nucleotide sequence comprises a nucleotide
- 5 sequence selected from the group consisting of the sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:5), Figure 5 (SEQ ID NO:7), Figure 8 (SEQ ID NO:13), Figure 11 (SEQ ID NO:19), Figure 14 (SEQ ID NO:22), Figure 17 (SEQ ID NO:27), Figure 19 (SEQ ID NO:29), Figure 22 (SEQ ID NO:32), Figure 24 (SEQ ID NO:35), Figure 26 (SEQ ID NO:40), Figure 29 (SEQ ID NO:46), Figure 31 (SEQ ID NO:51), Figure 33 (SEQ ID NO:56), Figure 35 (SEQ ID NO:61), Figure 37 (SEQ ID NO:66), Figure 40 (SEQ ID NO:72),
- 10 Figure 46 (SEQ ID NO:83), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:96), Figure 52 (SEQ ID NO:98), Figure 56 (SEQ ID NO:102), Figure 63 (SEQ ID NO:112), Figure 65 (SEQ ID NO:114), Figure 67 (SEQ ID NO:116), Figure 69 (SEQ ID NO:118), Figure 71 (SEQ ID NO:123), Figure 73 (SEQ ID NO:128), Figure 75 (SEQ ID NO:134), Figure 78 (SEQ ID NO:137), Figure 82 (SEQ ID NO:145), Figure 84 (SEQ ID NO:147), Figure 87 (SEQ ID NO:150), Figure 89 (SEQ ID NO:152), Figure 92 (SEQ ID NO:155), Figure 94
- 15 (SEQ ID NO:157), Figure 96 (SEQ ID NO:159), Figure 98 (SEQ ID NO:164), Figure 100 (SEQ ID NO:166), Figure 102 (SEQ ID NO:168), Figure 104 (SEQ ID NO:170), Figure 108 (SEQ ID NO:174), Figure 110 (SEQ ID NO:176), Figure 112 (SEQ ID NO:178), Figure 114 (SEQ ID NO:180), Figure 116 (SEQ ID NO:182), Figure 119 (SEQ ID NO:188), Figure 121 (SEQ ID NO:193), Figure 124 (SEQ ID NO:196), Figure 126 (SEQ ID NO:198), Figure 128 (SEQ ID NO:200), Figure 130 (SEQ ID NO:202), Figure 132 (SEQ ID NO:204),
- 20 Figure 134 (SEQ ID NO:206), Figure 136 (SEQ ID NO:208), Figure 138 (SEQ ID NO:210), Figure 140 (SEQ ID NO:212), Figure 143 (SEQ ID NO:215), Figure 146 (SEQ ID NO:218), Figure 148 (SEQ ID NO:220), Figure 150 (SEQ ID NO:222), Figure 152 (SEQ ID NO:224), Figure 154 (SEQ ID NO:226), Figure 156 (SEQ ID NO:228), Figure 158 (SEQ ID NO:230), Figure 160 (SEQ ID NO:235), Figure 162 (SEQ ID NO:240), Figure 164 (SEQ ID NO:245), Figure 166 (SEQ ID NO:247), Figure 168 (SEQ ID NO:249), Figure 170 (SEQ
- 25 ID NO:252), Figure 173 (SEQ ID NO:255), Figure 175 (SEQ ID NO:257), Figure 177 (SEQ ID NO:259), Figure 179 (SEQ ID NO:261), Figure 181 (SEQ ID NO:263), Figure 183 (SEQ ID NO:265), Figure 185 (SEQ ID NO:267), Figure 187 (SEQ ID NO:269), Figure 189 (SEQ ID NO:271), Figure 191 (SEQ ID NO:273), Figure 193 (SEQ ID NO:275), Figure 195 (SEQ ID NO:277), Figure 197 (SEQ ID NO:280), Figure 199 (SEQ ID NO:282), Figure 201 (SEQ ID NO:284), Figure 203 (SEQ ID NO:286), Figure 205 (SEQ ID NO:288),
- 30 Figure 207 (SEQ ID NO:290), Figure 209 (SEQ ID NO:292), Figure 211 (SEQ ID NO:294), Figure 213 (SEQ ID NO:296), Figure 215 (SEQ ID NO:298), Figure 217 (SEQ ID NO:300), Figure 219 (SEQ ID NO:302), Figure 225 (SEQ ID NO:308), Figure 227 (SEQ ID NO:313), Figure 229 (SEQ ID NO:318), Figure 232 (SEQ ID NO:325), Figure 234 (SEQ ID NO:333), Figure 237 (SEQ ID NO:339), Figure 239 (SEQ ID NO:344), Figure 241 (SEQ ID NO:346), Figure 243 (SEQ ID NO:348), Figure 245 (SEQ ID NO:350), Figure 247 (SEQ
- 35 ID NO:352), Figure 249 (SEQ ID NO:354), Figure 251 (SEQ ID NO:356), Figure 253 (SEQ ID NO:358), Figure 255 (SEQ ID NO:360), Figure 257 (SEQ ID NO:362), Figure 259 (SEQ ID NO:364), Figure 261 (SEQ ID NO:366), Figure 263 (SEQ ID NO:368), Figure 265 (SEQ ID NO:370), Figure 267 (SEQ ID NO:372),

Figure 269 (SEQ ID NO:374), Figure 271 (SEQ ID NO:376), Figure 273 (SEQ ID NO:378), Figure 275 (SEQ ID NO:380), Figure 277 (SEQ ID NO:386), Figure 279 (SEQ ID NO:388), Figure 281 (SEQ ID NO:393), Figure 283 (SEQ ID NO:398), Figure 285 (SEQ ID NO:400), Figure 287 (SEQ ID NO:402), Figure 289 (SEQ ID NO:407), Figure 291 (SEQ ID NO:409), Figure 293 (SEQ ID NO:411), Figure 295 (SEQ ID NO:413), Figure 297 (SEQ ID NO:415), Figure 299 (SEQ ID NO:417), Figure 301 (SEQ ID NO:419), Figure 303 (SEQ ID NO:421) and Figure 305 (SEQ ID NO:423).

3. The nucleic acid of Claim 1, wherein said nucleotide sequence comprises a nucleotide sequence selected from the group consisting of the full-length coding sequence of the sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:5), Figure 5 (SEQ ID NO:7), Figure 8 (SEQ ID NO:13), Figure 11 (SEQ ID NO:19), Figure 14 (SEQ ID NO:22), Figure 17 (SEQ ID NO:27), Figure 19 (SEQ ID NO:29), Figure 22 (SEQ ID NO:32), Figure 24 (SEQ ID NO:35), Figure 26 (SEQ ID NO:40), Figure 29 (SEQ ID NO:46), Figure 31 (SEQ ID NO:51), Figure 33 (SEQ ID NO:56), Figure 35 (SEQ ID NO:61), Figure 37 (SEQ ID NO:66), Figure 40 (SEQ ID NO:72), Figure 46 (SEQ ID NO:83), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:96), Figure 52 (SEQ ID NO:98), Figure 56 (SEQ ID NO:102), Figure 63 (SEQ ID NO:112), Figure 65 (SEQ ID NO:114), Figure 67 (SEQ ID NO:116), Figure 69 (SEQ ID NO:118), Figure 71 (SEQ ID NO:123), Figure 73 (SEQ ID NO:128), Figure 75 (SEQ ID NO:134), Figure 78 (SEQ ID NO:137), Figure 82 (SEQ ID NO:145), Figure 84 (SEQ ID NO:147), Figure 87 (SEQ ID NO:150), Figure 89 (SEQ ID NO:152), Figure 92 (SEQ ID NO:155), Figure 94 (SEQ ID NO:157), Figure 96 (SEQ ID NO:159), Figure 98 (SEQ ID NO:164), Figure 100 (SEQ ID NO:166), Figure 102 (SEQ ID NO:168), Figure 104 (SEQ ID NO:170), Figure 108 (SEQ ID NO:174), Figure 110 (SEQ ID NO:176), Figure 112 (SEQ ID NO:178), Figure 114 (SEQ ID NO:180), Figure 116 (SEQ ID NO:182), Figure 119 (SEQ ID NO:188), Figure 121 (SEQ ID NO:193), Figure 124 (SEQ ID NO:196), Figure 126 (SEQ ID NO:198), Figure 128 (SEQ ID NO:200), Figure 130 (SEQ ID NO:202), Figure 132 (SEQ ID NO:204), Figure 134 (SEQ ID NO:206), Figure 136 (SEQ ID NO:208), Figure 138 (SEQ ID NO:210), Figure 140 (SEQ ID NO:212), Figure 143 (SEQ ID NO:215), Figure 146 (SEQ ID NO:218), Figure 148 (SEQ ID NO:220), Figure 150 (SEQ ID NO:222), Figure 152 (SEQ ID NO:224), Figure 154 (SEQ ID NO:226), Figure 156 (SEQ ID NO:228), Figure 158 (SEQ ID NO:230), Figure 160 (SEQ ID NO:235), Figure 162 (SEQ ID NO:240), Figure 164 (SEQ ID NO:245), Figure 166 (SEQ ID NO:247), Figure 168 (SEQ ID NO:249), Figure 170 (SEQ ID NO:252), Figure 173 (SEQ ID NO:255), Figure 175 (SEQ ID NO:257), Figure 177 (SEQ ID NO:259), Figure 179 (SEQ ID NO:261), Figure 181 (SEQ ID NO:263), Figure 183 (SEQ ID NO:265), Figure 185 (SEQ ID NO:267), Figure 187 (SEQ ID NO:269), Figure 189 (SEQ ID NO:271), Figure 191 (SEQ ID NO:273), Figure 193 (SEQ ID NO:275), Figure 195 (SEQ ID NO:277), Figure 197 (SEQ ID NO:280), Figure 199 (SEQ ID NO:282), Figure 201 (SEQ ID NO:284), Figure 203 (SEQ ID NO:286), Figure 205 (SEQ ID NO:288), Figure 207 (SEQ ID NO:290), Figure 209 (SEQ ID NO:292), Figure 211 (SEQ ID NO:294), Figure 213 (SEQ ID NO:296), Figure 215 (SEQ ID NO:298), Figure 217 (SEQ ID NO:300), Figure 219 (SEQ ID NO:302), Figure 225 (SEQ ID NO:308), Figure 227 (SEQ ID NO:313), Figure 229 (SEQ ID NO:318), Figure 232 (SEQ ID NO:325), Figure 234 (SEQ ID NO:333), Figure 237 (SEQ ID NO:339), Figure 239 (SEQ ID NO:344), Figure 241 (SEQ ID NO:346), Figure 243 (SEQ ID NO:348), Figure 245 (SEQ ID

NO:350), Figure 247 (SEQ ID NO:352), Figure 249 (SEQ ID NO:354), Figure 251 (SEQ ID NO:356), Figure 253 (SEQ ID NO:358), Figure 255 (SEQ ID NO:360), Figure 257 (SEQ ID NO:362), Figure 259 (SEQ ID NO:364), Figure 261 (SEQ ID NO:366), Figure 263 (SEQ ID NO:368), Figure 265 (SEQ ID NO:370), Figure 267 (SEQ ID NO:372), Figure 269 (SEQ ID NO:374), Figure 271 (SEQ ID NO:376), Figure 273 (SEQ ID NO:378), Figure 275 (SEQ ID NO:380), Figure 277 (SEQ ID NO:386), Figure 279 (SEQ ID NO:388), Figure 281 (SEQ ID NO:393), Figure 283 (SEQ ID NO:398), Figure 285 (SEQ ID NO:400), Figure 287 (SEQ ID NO:402), Figure 289 (SEQ ID NO:407), Figure 291 (SEQ ID NO:409), Figure 293 (SEQ ID NO:411), Figure 295 (SEQ ID NO:413), Figure 297 (SEQ ID NO:415), Figure 299 (SEQ ID NO:417), Figure 301 (SEQ ID NO:419), Figure 303 (SEQ ID NO:421) or Figure 305 (SEQ ID NO:423).

10           4.       Isolated nucleic acid which comprises the full-length coding sequence of the DNA deposited under any ATCC accession number shown in Table 2.

5                   5.       A vector comprising the nucleic acid of Claim 1.

15           6.       The vector of Claim 5 operably linked to control sequences recognized by a host cell transformed with the vector.

7.       A host cell comprising the vector of Claim 5.

20           8.       The host cell of Claim 7 wherein said cell is a CHO cell.

9.       The host cell of Claim 7 wherein said cell is an *E. coli*.

10.       The host cell of Claim 7 wherein said cell is a yeast cell.

25           11.       A process for producing a PRO polypeptides comprising culturing the host cell of Claim 7 under conditions suitable for expression of said PRO polypeptide and recovering said PRO polypeptide from the cell culture.

30           12.       Isolated PRO polypeptide having at least 80% sequence identity to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:6), Figure 6 (SEQ ID NO:8), Figure 9 (SEQ ID NO:14), Figure 12 (SEQ ID NO:20), Figure 15 (SEQ ID NO:23), Figure 18 (SEQ ID NO:28), Figure 20 (SEQ ID NO:30), Figure 23 (SEQ ID NO:33), Figure 25 (SEQ ID NO:36), Figure 27 (SEQ ID NO:41), Figure 30 (SEQ ID NO:47), Figure 32 (SEQ ID NO:52), Figure 34 (SEQ ID NO:57), Figure 36 (SEQ ID NO:62), Figure 38 (SEQ ID NO:67), Figure 41 (SEQ ID NO:73), Figure 47 (SEQ ID NO:84), Figure 49 (SEQ ID NO:95), Figure 51 (SEQ ID NO:97), Figure 53 (SEQ ID NO:99), Figure 57 (SEQ ID NO:103), Figure 64 (SEQ ID NO:113), Figure 66 (SEQ ID NO:115), Figure 68

(SEQ ID NO:117), Figure 70 (SEQ ID NO:119), Figure 72 (SEQ ID NO:124), Figure 74 (SEQ ID NO:129), Figure 76 (SEQ ID NO:135), Figure 79 (SEQ ID NO:138), Figure 83 (SEQ ID NO:146), Figure 85 (SEQ ID NO:148), Figure 88 (SEQ ID NO:151), Figure 90 (SEQ ID NO:153), Figure 93 (SEQ ID NO:156), Figure 95 (SEQ ID NO:158), Figure 97 (SEQ ID NO:160), Figure 99 (SEQ ID NO:165), Figure 101 (SEQ ID NO:167), Figure 103 (SEQ ID NO:169), Figure 105 (SEQ ID NO:171), Figure 109 (SEQ ID NO:175), Figure 111 (SEQ ID NO:177), Figure 113 (SEQ ID NO:179), Figure 115 (SEQ ID NO:181), Figure 117 (SEQ ID NO:183), Figure 120 (SEQ ID NO:189), Figure 122 (SEQ ID NO:194), Figure 125 (SEQ ID NO:197), Figure 127 (SEQ ID NO:199), Figure 129 (SEQ ID NO:201), Figure 131 (SEQ ID NO:203), Figure 133 (SEQ ID NO:205), Figure 135 (SEQ ID NO:207), Figure 137 (SEQ ID NO:209), Figure 139 (SEQ ID NO:211), Figure 141 (SEQ ID NO:213), Figure 144 (SEQ ID NO:216), Figure 147 (SEQ ID NO:219), Figure 149 (SEQ ID NO:221), Figure 151 (SEQ ID NO:223), Figure 153 (SEQ ID NO:225), Figure 155 (SEQ ID NO:227), Figure 157 (SEQ ID NO:229), Figure 159 (SEQ ID NO:231), Figure 161 (SEQ ID NO:236), Figure 163 (SEQ ID NO:241), Figure 165 (SEQ ID NO:246), Figure 167 (SEQ ID NO:248), Figure 169 (SEQ ID NO:250), Figure 171 (SEQ ID NO:253), Figure 174 (SEQ ID NO:256), Figure 176 (SEQ ID NO:258), Figure 178 (SEQ ID NO:260), Figure 180 (SEQ ID NO:262), Figure 182 (SEQ ID NO:264), Figure 184 (SEQ ID NO:266), Figure 186 (SEQ ID NO:268), Figure 188 (SEQ ID NO:270), Figure 190 (SEQ ID NO:272), Figure 192 (SEQ ID NO:274), Figure 194 (SEQ ID NO:276), Figure 196 (SEQ ID NO:278), Figure 198 (SEQ ID NO:281), Figure 200 (SEQ ID NO:283), Figure 202 (SEQ ID NO:285), Figure 204 (SEQ ID NO:287), Figure 206 (SEQ ID NO:289), Figure 208 (SEQ ID NO:291), Figure 210 (SEQ ID NO:293), Figure 212 (SEQ ID NO:295), Figure 214 (SEQ ID NO:297), Figure 216 (SEQ ID NO:299), Figure 218 (SEQ ID NO:301), Figure 220 (SEQ ID NO:303), Figure 222 (SEQ ID NO:309), Figure 224 (SEQ ID NO:314), Figure 226 (SEQ ID NO:319), Figure 228 (SEQ ID NO:326), Figure 230 (SEQ ID NO:334), Figure 232 (SEQ ID NO:340), Figure 234 (SEQ ID NO:345), Figure 236 (SEQ ID NO:347), Figure 238 (SEQ ID NO:349), Figure 240 (SEQ ID NO:351), Figure 242 (SEQ ID NO:353), Figure 244 (SEQ ID NO:355), Figure 246 (SEQ ID NO:357), Figure 248 (SEQ ID NO:359), Figure 250 (SEQ ID NO:361), Figure 252 (SEQ ID NO:363), Figure 254 (SEQ ID NO:365), Figure 256 (SEQ ID NO:367), Figure 258 (SEQ ID NO:369), Figure 260 (SEQ ID NO:371), Figure 262 (SEQ ID NO:373), Figure 264 (SEQ ID NO:375), Figure 266 (SEQ ID NO:377), Figure 268 (SEQ ID NO:379), Figure 270 (SEQ ID NO:381), Figure 272 (SEQ ID NO:387), Figure 274 (SEQ ID NO:389), Figure 276 (SEQ ID NO:394), Figure 278 (SEQ ID NO:399), Figure 280 (SEQ ID NO:401), Figure 282 (SEQ ID NO:403), Figure 284 (SEQ ID NO:408), Figure 286 (SEQ ID NO:410), Figure 288 (SEQ ID NO:412), Figure 290 (SEQ ID NO:414), Figure 292 (SEQ ID NO:416), Figure 294 (SEQ ID NO:418), Figure 296 (SEQ ID NO:420), Figure 298 (SEQ ID NO:422) and Figure 300 (SEQ ID NO:424).

13. Isolated PRO polypeptide having at least 80% sequence identity to the amino acid sequence encoded by a nucleic acid molecule deposited under any ATCC accession number shown in Table 2.

14. A chimeric molecule comprising a polypeptide according to Claim 12 fused to a heterologous amino acid sequence.



15. The chimeric molecule of Claim 14 wherein said heterologous amino acid sequence is an epitope tag sequence.

16. The chimeric molecule of Claim 14 wherein said heterologous amino acid sequence is a Fc region of an immunoglobulin.

5

17. An antibody which specifically binds to a PRO polypeptide according to Claim 12.

18. The antibody of Claim 17 wherein said antibody is a monoclonal antibody.

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19. The antibody of Claim 17 wherein said antibody is a humanized antibody.

20. The antibody of Claim 17 wherein said antibody is an antibody fragment.

21. An isolated nucleic acid molecule which has at least 80% sequence identity to a nucleic acid  
15 which comprises a nucleotide sequence selected from the group consisting of that shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:5), Figure 5 (SEQ ID NO:7), Figure 8 (SEQ ID NO:13), Figure 11 (SEQ ID NO:19), Figure 14 (SEQ ID NO:22), Figure 17 (SEQ ID NO:27), Figure 19 (SEQ ID NO:29), Figure 22 (SEQ ID NO:32), Figure 24 (SEQ ID NO:35), Figure 26 (SEQ ID NO:40), Figure 29 (SEQ ID NO:46), Figure 31 (SEQ ID NO:51), Figure 33 (SEQ ID NO:56), Figure 35 (SEQ ID NO:61), Figure 37 (SEQ ID NO:66), Figure  
20 40 (SEQ ID NO:72), Figure 46 (SEQ ID NO:83), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:96), Figure 52 (SEQ ID NO:98), Figure 56 (SEQ ID NO:102), Figure 63 (SEQ ID NO:112), Figure 65 (SEQ ID NO:114), Figure 67 (SEQ ID NO:116), Figure 69 (SEQ ID NO:118), Figure 71 (SEQ ID NO:123), Figure 73 (SEQ ID NO:128), Figure 75 (SEQ ID NO:134), Figure 78 (SEQ ID NO:137), Figure 82 (SEQ ID NO:145), Figure 84 (SEQ ID NO:147), Figure 87 (SEQ ID NO:150), Figure 89 (SEQ ID NO:152), Figure 92 (SEQ ID NO:155), Figure 94 (SEQ ID NO:157), Figure 96 (SEQ ID NO:159), Figure 98 (SEQ ID NO:164), Figure 100 (SEQ ID NO:166), Figure 102 (SEQ ID NO:168), Figure 104 (SEQ ID NO:170), Figure 108 (SEQ ID NO:174), Figure 110 (SEQ ID NO:176), Figure 112 (SEQ ID NO:178), Figure 114 (SEQ ID NO:180), Figure 116 (SEQ ID NO:182), Figure 119 (SEQ ID NO:188), Figure 121 (SEQ ID NO:193), Figure 124 (SEQ ID NO:196), Figure 126 (SEQ ID NO:198), Figure 128 (SEQ ID NO:200), Figure 130 (SEQ ID NO:202), Figure  
30 132 (SEQ ID NO:204), Figure 134 (SEQ ID NO:206), Figure 136 (SEQ ID NO:208), Figure 138 (SEQ ID NO:210), Figure 140 (SEQ ID NO:212), Figure 143 (SEQ ID NO:215), Figure 146 (SEQ ID NO:218), Figure 148 (SEQ ID NO:220), Figure 150 (SEQ ID NO:222), Figure 152 (SEQ ID NO:224), Figure 154 (SEQ ID NO:226), Figure 156 (SEQ ID NO:228), Figure 158 (SEQ ID NO:230), Figure 160 (SEQ ID NO:235), Figure 162 (SEQ ID NO:240), Figure 164 (SEQ ID NO:245), Figure 166 (SEQ ID NO:247), Figure 168 (SEQ ID NO:249), Figure 170 (SEQ ID NO:252), Figure 173 (SEQ ID NO:255), Figure 175 (SEQ ID NO:257), Figure 177 (SEQ ID NO:259), Figure 179 (SEQ ID NO:261), Figure 181 (SEQ ID NO:263), Figure 183 (SEQ ID NO:265), Figure 185 (SEQ ID NO:267), Figure 187 (SEQ ID NO:269), Figure 189 (SEQ ID NO:271), Figure  
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191 (SEQ ID NO:273), Figure 193 (SEQ ID NO:275), Figure 195 (SEQ ID NO:277), Figure 197 (SEQ ID NO:280), Figure 199 (SEQ ID NO:282), Figure 201 (SEQ ID NO:284), Figure 203 (SEQ ID NO:286), Figure 205 (SEQ ID NO:288), Figure 207 (SEQ ID NO:290), Figure 209 (SEQ ID NO:292), Figure 211 (SEQ ID NO:294), Figure 213 (SEQ ID NO:296), Figure 215 (SEQ ID NO:298), Figure 217 (SEQ ID NO:300), Figure 219 (SEQ ID NO:302), Figure 225 (SEQ ID NO:308), Figure 227 (SEQ ID NO:313), Figure 229 (SEQ ID NO:318), Figure 232 (SEQ ID NO:325), Figure 234 (SEQ ID NO:333), Figure 237 (SEQ ID NO:339), Figure 239 (SEQ ID NO:344), Figure 241 (SEQ ID NO:346), Figure 243 (SEQ ID NO:348), Figure 245 (SEQ ID NO:350), Figure 247 (SEQ ID NO:352), Figure 249 (SEQ ID NO:354), Figure 251 (SEQ ID NO:356), Figure 253 (SEQ ID NO:358), Figure 255 (SEQ ID NO:360), Figure 257 (SEQ ID NO:362), Figure 259 (SEQ ID NO:364), Figure 261 (SEQ ID NO:366), Figure 263 (SEQ ID NO:368), Figure 265 (SEQ ID NO:370), Figure 267 (SEQ ID NO:372), Figure 269 (SEQ ID NO:374), Figure 271 (SEQ ID NO:376), Figure 273 (SEQ ID NO:378), Figure 275 (SEQ ID NO:380), Figure 277 (SEQ ID NO:386), Figure 279 (SEQ ID NO:388), Figure 281 (SEQ ID NO:393), Figure 283 (SEQ ID NO:398), Figure 285 (SEQ ID NO:400), Figure 287 (SEQ ID NO:402), Figure 289 (SEQ ID NO:407), Figure 291 (SEQ ID NO:409), Figure 293 (SEQ ID NO:411), Figure 295 (SEQ ID NO:413), Figure 297 (SEQ ID NO:415), Figure 299 (SEQ ID NO:417), Figure 301 (SEQ ID NO:419), Figure 303 (SEQ ID NO:421) and Figure 305 (SEQ ID NO:423).

22. An isolated nucleic acid molecule which has at least 80% sequence identity to the full-length coding sequence of a nucleotide sequence selected from the group consisting of that shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:5), Figure 5 (SEQ ID NO:7), Figure 8 (SEQ ID NO:13), Figure 11 (SEQ ID NO:19), Figure 14 (SEQ ID NO:22), Figure 17 (SEQ ID NO:27), Figure 19 (SEQ ID NO:29), Figure 22 (SEQ ID NO:32), Figure 24 (SEQ ID NO:35), Figure 26 (SEQ ID NO:40), Figure 29 (SEQ ID NO:46), Figure 31 (SEQ ID NO:51), Figure 33 (SEQ ID NO:56), Figure 35 (SEQ ID NO:61), Figure 37 (SEQ ID NO:66), Figure 40 (SEQ ID NO:72), Figure 46 (SEQ ID NO:83), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:96), Figure 52 (SEQ ID NO:98), Figure 56 (SEQ ID NO:102), Figure 63 (SEQ ID NO:112), Figure 65 (SEQ ID NO:114), Figure 67 (SEQ ID NO:116), Figure 69 (SEQ ID NO:118), Figure 71 (SEQ ID NO:123), Figure 73 (SEQ ID NO:128), Figure 75 (SEQ ID NO:134), Figure 78 (SEQ ID NO:137), Figure 82 (SEQ ID NO:145), Figure 84 (SEQ ID NO:147), Figure 87 (SEQ ID NO:150), Figure 89 (SEQ ID NO:152), Figure 92 (SEQ ID NO:155), Figure 94 (SEQ ID NO:157), Figure 96 (SEQ ID NO:159), Figure 98 (SEQ ID NO:164), Figure 100 (SEQ ID NO:166), Figure 102 (SEQ ID NO:168), Figure 104 (SEQ ID NO:170), Figure 108 (SEQ ID NO:174), Figure 110 (SEQ ID NO:176), Figure 112 (SEQ ID NO:178), Figure 114 (SEQ ID NO:180), Figure 116 (SEQ ID NO:182), Figure 119 (SEQ ID NO:188), Figure 121 (SEQ ID NO:193), Figure 124 (SEQ ID NO:196), Figure 126 (SEQ ID NO:198), Figure 128 (SEQ ID NO:200), Figure 130 (SEQ ID NO:202), Figure 132 (SEQ ID NO:204), Figure 134 (SEQ ID NO:206), Figure 136 (SEQ ID NO:208), Figure 138 (SEQ ID NO:210), Figure 140 (SEQ ID NO:212), Figure 143 (SEQ ID NO:215), Figure 146 (SEQ ID NO:218), Figure 148 (SEQ ID NO:220), Figure 150 (SEQ ID NO:222), Figure 152 (SEQ ID NO:224), Figure 154 (SEQ ID NO:226), Figure 156 (SEQ ID NO:228), Figure 158 (SEQ ID NO:230), Figure 160 (SEQ ID NO:235), Figure 162 (SEQ ID NO:240), Figure 164 (SEQ ID NO:245), Figure 166 (SEQ ID NO:247), Figure 168 (SEQ ID

NO:249), Figure 170 (SEQ ID NO:252), Figure 173 (SEQ ID NO:255), Figure 175 (SEQ ID NO:257), Figure 177 (SEQ ID NO:259), Figure 179 (SEQ ID NO:261), Figure 181 (SEQ ID NO:263), Figure 183 (SEQ ID NO:265), Figure 185 (SEQ ID NO:267), Figure 187 (SEQ ID NO:269), Figure 189 (SEQ ID NO:271), Figure 191 (SEQ ID NO:273), Figure 193 (SEQ ID NO:275), Figure 195 (SEQ ID NO:277), Figure 197 (SEQ ID NO:280), Figure 199 (SEQ ID NO:282), Figure 201 (SEQ ID NO:284), Figure 203 (SEQ ID NO:286), Figure 205 (SEQ ID NO:288), Figure 207 (SEQ ID NO:290), Figure 209 (SEQ ID NO:292), Figure 211 (SEQ ID NO:294), Figure 213 (SEQ ID NO:296), Figure 215 (SEQ ID NO:298), Figure 217 (SEQ ID NO:300), Figure 219 (SEQ ID NO:302), Figure 225 (SEQ ID NO:308), Figure 227 (SEQ ID NO:313), Figure 229 (SEQ ID NO:318), Figure 232 (SEQ ID NO:325), Figure 234 (SEQ ID NO:333), Figure 237 (SEQ ID NO:339), Figure 239 (SEQ ID NO:344), Figure 241 (SEQ ID NO:346), Figure 243 (SEQ ID NO:348), Figure 245 (SEQ ID NO:350), Figure 247 (SEQ ID NO:352), Figure 249 (SEQ ID NO:354), Figure 251 (SEQ ID NO:356), Figure 253 (SEQ ID NO:358), Figure 255 (SEQ ID NO:360), Figure 257 (SEQ ID NO:362), Figure 259 (SEQ ID NO:364), Figure 261 (SEQ ID NO:366), Figure 263 (SEQ ID NO:368), Figure 265 (SEQ ID NO:370), Figure 267 (SEQ ID NO:372), Figure 269 (SEQ ID NO:374), Figure 271 (SEQ ID NO:376), Figure 273 (SEQ ID NO:378), Figure 275 (SEQ ID NO:380), Figure 277 (SEQ ID NO:386), Figure 279 (SEQ ID NO:388), Figure 281 (SEQ ID NO:393), Figure 283 (SEQ ID NO:398), Figure 285 (SEQ ID NO:400), Figure 287 (SEQ ID NO:402), Figure 289 (SEQ ID NO:407), Figure 291 (SEQ ID NO:409), Figure 293 (SEQ ID NO:411), Figure 295 (SEQ ID NO:413), Figure 297 (SEQ ID NO:415), Figure 299 (SEQ ID NO:417), Figure 301 (SEQ ID NO:419), Figure 303 (SEQ ID NO:421) and Figure 305 (SEQ ID NO:423).

- 20           23.     An isolated extracellular domain of of PRO polypeptide.
24.     An isolated PRO polypeptide lacking its associated signal peptide.
25.     An isolated polypeptide having at least about 80% amino acid sequence identity to an  
25 extracellular domain of of PRO polypeptide.
26.     An isolated polypeptide having at least about 80% amino acid sequence identity to a PRO  
polypeptide lacking its associated signal peptide.

**FIGURE 177**

GTCGAATCCAAATCACTCATTGTGAAAGCTGAGCTCACAGCCGAATAAGCCACCATGAGGCT  
GTCAGTGTGTCTCCTGATGGTCTCGCTGGCCCTTTGCTGCTACCAGGCCCATGCTCTTGTCT  
GCCCAGCTGTTGCTTCTGAGATCACAGTCTTCTTATTCTTAAGTGACGCTGCGGTAAACCTC  
CAAGTTGCCAAACTTAATCCACCTCCAGAAGCTCTTGCAGCCAAGTTGGAAGTGAAGCACTG  
CACCGATCAGATATCTTTTAAGAAACGACTCTCATTGAAAAAGTCCTGGTGGAAATAGTGAA  
AAAATGTGGTGTGTGACATGTAAAAATGCTCAACCTGGTTTCAAAGTCTTTC AACGACACC  
CTGATCTTCACTAAAAATTGTAAAGGTTTCAACACGTTGCTTTAATAAATCACTTGCCCTGC

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**FIGURE 178**

MRLSVCLLMVSLALCCYQAHALVCPAVASEITVFLFLSDAAVNLQVAKLNPPPEALAAKLEV  
KHCTDQISFKKRLSLKKSWWK

(30) 60/088,742	10 Jun/juin 1998 (10.06.1998)	US	(30) 60/090,254	22 Jun/juin 1998 (22.06.1998)	US	(30) 60/091,478	2 Jul/juil 1998 (02.07.1998)	US
(30) 60/088,810	10 Jun/juin 1998 (10.06.1998)	US	(30) 60/090,355	23 Jun/juin 1998 (23.06.1998)	US	(30) 60/091,626	2 Jul/juil 1998 (02.07.1998)	US
(30) 60/088,811	10 Jun/juin 1998 (10.06.1998)	US	(30) 60/090,349	23 Jun/juin 1998 (23.06.1998)	US	(30) 60/091,628	2 Jul/juil 1998 (02.07.1998)	US
(30) 60/088,824	10 Jun/juin 1998 (10.06.1998)	US	(30) 60/090,429	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/091,633	2 Jul/juil 1998 (02.07.1998)	US
(30) 60/088,825	10 Jun/juin 1998 (10.06.1998)	US	(30) 60/090,431	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/091,646	2 Jul/juil 1998 (02.07.1998)	US
(30) 60/088,826	10 Jun/juin 1998 (10.06.1998)	US	(30) 60/090,435	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/091,673	2 Jul/juil 1998 (02.07.1998)	US
(30) 60/088,858	11 Jun/juin 1998 (11.06.1998)	US	(30) 60/090,444	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/091,978	7 Jul/juil 1998 (07.07.1998)	US
(30) 60/088,861	11 Jun/juin 1998 (11.06.1998)	US	(30) 60/090,445	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/091,982	7 Jul/juil 1998 (07.07.1998)	US
(30) 60/088,863	11 Jun/juin 1998 (11.06.1998)	US	(30) 60/090,461	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/092,182	9 Jul/juil 1998 (09.07.1998)	US
(30) 60/088,876	11 Jun/juin 1998 (11.06.1998)	US	(30) 60/090,472	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/092,472	10 Jul/juil 1998 (10.07.1998)	US
(30) 60/089,090	12 Jun/juin 1998 (12.06.1998)	US	(30) 60/090,535	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/093,339	20 Jul/juil 1998 (20.07.1998)	US
(30) 60/089,105	12 Jun/juin 1998 (12.06.1998)	US	(30) 60/090,538	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/094,651	30 Jul/juil 1998 (30.07.1998)	US
(30) 60/089,440	16 Jun/juin 1998 (16.06.1998)	US	(30) 60/090,540	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/095,282	4 Aug/août 1998 (04.08.1998)	US
(30) 60/089,512	16 Jun/juin 1998 (16.06.1998)	US	(30) 60/090,557	24 Jun/juin 1998 (24.06.1998)	US	(30) 60/095,285	4 Aug/août 1998 (04.08.1998)	US
(30) 60/089,514	16 Jun/juin 1998 (16.06.1998)	US	(30) 60/090,676	25 Jun/juin 1998 (25.06.1998)	US	(30) 60/095,301	4 Aug/août 1998 (04.08.1998)	US
(30) 60/089,532	17 Jun/juin 1998 (17.06.1998)	US	(30) 60/090,678	25 Jun/juin 1998 (25.06.1998)	US	(30) 60/095,302	4 Aug/août 1998 (04.08.1998)	US
(30) 60/089,538	17 Jun/juin 1998 (17.06.1998)	US	(30) 60/090,688	25 Jun/juin 1998 (25.06.1998)	US	(30) 60/095,318	4 Aug/août 1998 (04.08.1998)	US
(30) 60/089,598	17 Jun/juin 1998 (17.06.1998)	US	(30) 60/090,690	25 Jun/juin 1998 (25.06.1998)	US	(30) 60/095,321	4 Aug/août 1998 (04.08.1998)	US
(30) 60/089,599	17 Jun/juin 1998 (17.06.1998)	US	(30) 60/090,691	25 Jun/juin 1998 (25.06.1998)	US	(30) 60/095,325	4 Aug/août 1998 (04.08.1998)	US
(30) 60/089,600	17 Jun/juin 1998 (17.06.1998)	US	(30) 60/090,694	25 Jun/juin 1998 (25.06.1998)	US	(30) 60/095,916	10 Aug/août 1998 (10.08.1998)	US
(30) 60/089,653	17 Jun/juin 1998 (17.06.1998)	US	(30) 60/090,695	25 Jun/juin 1998 (25.06.1998)	US	(30) 60/095,929	10 Aug/août 1998 (10.08.1998)	US
(30) 60/089,801	18 Jun/juin 1998 (18.06.1998)	US	(30) 60/090,696	25 Jun/juin 1998 (25.06.1998)	US	(30) 60/096,012	10 Aug/août 1998 (10.08.1998)	US
(30) 60/089,907	18 Jun/juin 1998 (18.06.1998)	US	(30) 60/090,862	26 Jun/juin 1998 (26.06.1998)	US	(30) 60/096,143	11 Aug/août 1998 (11.08.1998)	US
(30) 60/089,908	18 Jun/juin 1998 (18.06.1998)	US	(30) 60/090,863	26 Jun/juin 1998 (26.06.1998)	US	(30) 60/096,146	11 Aug/août 1998 (11.08.1998)	US
(30) 60/089,947	19 Jun/juin 1998 (19.06.1998)	US	(30) 60/091,358	1 Jul/juil 1998 (01.07.1998)	US	(30) 60/096,329	12 Aug/août 1998 (12.08.1998)	US
(30) 60/089,948	19 Jun/juin 1998 (19.06.1998)	US	(30) 60/091,360	1 Jul/juil 1998 (01.07.1998)	US	(30) 60/096,757	17 Aug/août 1998 (17.08.1998)	US
(30) 60/089,952	19 Jun/juin 1998 (19.06.1998)	US	(30) 60/091,544	1 Jul/juil 1998 (01.07.1998)	US	(30) 60/096,766	17 Aug/août 1998 (17.08.1998)	US
(30) 60/090,246	22 Jun/juin 1998 (22.06.1998)	US	(30) 60/091,486	2 Jul/juil 1998 (02.07.1998)	US	(30) 60/096,768	17 Aug/août 1998 (17.08.1998)	US
(30) 60/090,252	22 Jun/juin 1998 (22.06.1998)	US	(30) 60/091,519	2 Jul/juil 1998 (02.07.1998)	US	(30) 60/096,773	17 Aug/août 1998 (17.08.1998)	US
						(30) 60/096,791	17 Aug/août 1998 (17.08.1998)	US

(30) 60/096,867	17 Aug/aout 1998 (17.08.1998)	US
(30) 60/096,891	17 Aug/aout 1998 (17.08.1998)	US
(30) 60/096,894	17 Aug/aout 1998 (17.08.1998)	US
(30) 60/096,895	17 Aug/aout 1998 (17.08.1998)	US
(30) 60/096,897	17 Aug/aout 1998 (17.08.1998)	US
(30) 60/096,949	18 Aug/aout 1998 (18.08.1998)	US
(30) 60/096,950	18 Aug/aout 1998 (18.08.1998)	US
(30) 60/096,959	18 Aug/aout 1998 (18.08.1998)	US
(30) 60/096,960	18 Aug/aout 1998 (18.08.1998)	US
(30) 60/097,022	18 Aug/aout 1998 (18.08.1998)	US
(30) 60/097,141	19 Aug/aout 1998 (19.08.1998)	US
(30) 60/097,218	20 Aug/aout 1998 (20.08.1998)	US
(30) 60/097,661	24 Aug/aout 1998 (24.08.1998)	US
(30) 60/097,951	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/097,952	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/097,954	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/097,955	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/097,971	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/097,974	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/097,978	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/097,979	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/097,986	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/098,014	26 Aug/aout 1998 (26.08.1998)	US
(30) 60/098,525	31 Aug/aout 1998 (31.08.1998)	US
(30) 60/100,634	16 Sep/sep 1998 (16.09.1998)	US
(30) 60/115,565	12 Jan/jan 1999 (12.01.1999)	US